RA
790.6
U559
1975
v.1

ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF MENTAL HEALTH
Fiscal Year 1975
Volume I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

Library, Acquisitions Unit
National Institutes of Health
Building 10
Bethesda, Maryland 20014

ANNUAL REPORT of program activities

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

U S NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1974 - June 30, 1975

VOLUME I

Summary Statements

RA
790.6
U559
1975
v.1

ANNUAL REPORT
MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research
NATIONAL INSTITUTE OF MENTAL HEALTH

July 1, 1974 - June 30, 1975

TABLE OF CONTENTS

VOLUME I - SUMMARY STATEMENTS

Page

Director, Mental Health Intramural Research Program.................... 1

Director, Division of Clinical and Behavioral Research................. 17

Acting Director, Division of Special Mental Health Research............ 21

DIVISION OF CLINICAL AND BEHAVIORAL RESEARCH

Adult Psychiatry Branch.. 25

Laboratory of Clinical Science..................................... 29

Laboratory of Developmental Psychology............................. 43

Laboratory of Psychology and Psychopathology........................ 53

Laboratory of Socio-Environmental Studies........................... 69

DIVISION OF BIOLOGICAL AND BIOCHEMICAL RESEARCH

Laboratory of Brain Evolution and Behavior.......................... 79

Laboratory of Cerebral Metabolism.................................. 83

Laboratory of General and Comparative Biochemistry.................. 89

Laboratory of Neurobiology... 93

Laboratory of Neurochemistry....................................... 97

Laboratory of Neurophysiology...................................... 101

DIVISION OF SPECIAL MENTAL HEALTH RESEARCH

Page

Office of the Director

Section on Neurochemistry 113

Laboratory of Neuropharmacology 117

Laboratory of Clinical Psychopharmacology 137

Laboratory of Preclinical Pharmacology 141

Office of the Director - Intramural Research Program

Section on Technical Development 155

ANNUAL REPORT
# of the
DIRECTOR, MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM
NATIONAL INSTITUTE OF MENTAL HEALTH
July 1, 1974 - June 30, 1975

John C. Eberhart, Ph.D.

One ventures to hope that the year just completed may eventually be seen as the beginning of a new era at the NIH-NIMH. Such hopes should be softly voiced, lest they jinx the processes of institutional and political change, but it is worth noting the portents. If it seems odd to begin an annual report of an intramural program by talking about the promise of stability and effective leadership at higher bureaucratic levels, it only reflects my strong feeling that leadership within and above organizations is vital to their success.

For the first time in a long time today (August 4, 1975, two months later than my usual report) finds us with effective, I may even say outstanding, leadership in the offices of the Assistant Secretary for Health, the Director of NIH, and the Administrator of ADAMHA. After a long wait following the departure of Dr. Charles Edwards as Assistant Secretary for Health and Dr. Robert Stone as Director, NIH, Secretary Weinberger came to the NIH Reunion on April 19, 1975, and at that cheerful event in the circus tent forecast that on the following Monday Dr. Theodore Cooper would be nominated by President Ford as Assistant Secretary for Health, and Dr. Donald Fredrickson as Director of NIH. And on July 1, 1975, President Ford came to the Clinical Center and presided at the swearing in of these two ex-NHLI scientists. Mr. James D. Isbister was nominated as Administrator of ADAMHA a short time later; was confirmed by the Senate at the end of July, and will be sworn in by HEW Secretary David Mathews at the Parklawn Building on August 20.

The NIMH has enjoyed effective continuing leadership in Dr. Bertram Brown. Now, with the other key positions covered by able and experienced men, one has the right to be hopeful that the machinery of government in the health phase of this Department will run faster and more responsibly than at any time in the recent past. I am personally very pleased at all three appointments.

What will the new era bring? In health science no one can see 10 years ahead, let alone 20. The NIH-NIMH will undoubtedly be different in many important respects, but one hopes that they will be even stronger, will be well staffed with good men and women from as many ethnic groups as wish to engage in world class biomedical research, with faith in their missions and pride in their work. One hopes too that the decisions affecting these agencies will be made more often than they now are with the agencies' full participation, and that the Department of HEW (or of Health) will delegate ample authority to the responsible agency administrators. The contents of the agency programs, the tasks to be accomplished, can well be decided by these organizations in the years ahead as old problems are solved and new problems present themselves. My wish for the new era I believe I see is that the NIH and NIMH be effective, that they pursue their missions with vigor, and that they be successful in accomplishing their missions. The problems of health and illness deserve no less of an organizational effort; the best of staffs needs no less of an organizational home.

The Intramural Program's 14 Laboratories have carried out their usual excellent research programs over the past year. The Chief of each Laboratory has summarized its achievements in the remainder of this Annual Report, and I shall not attempt any further summary here. I do want, however, to call attention to one discovery of fundamental importance from the Laboratory of Cerebral Metabolism, one which has major implications for the study of brain function. Dr. Louis Sokoloff, Chief of the Laboratory, describes in his summary and project report the discovery and demonstration, climaxing 15 years of work, that (1) in the brain, as in the other bodily tissues, functional activity of any part is paralleled by metabolic activity, and glucose consumption is a measure of the level of metabolic activity; (2) although there is no known way of measuring glucose consumption directly, he has developed an accurate indirect measure through the use of a radioactive analogue of glucose--[^{14}C]deoxyglucose--which, to quote Dr. Sokoloff, "...is partially metabolized just like glucose but then stops and accumulates in the cerebral tissues in a quantitative relationship to the rate of glucose consumption... One of the features of this new method is the use of quantitative autoradiography to measure tissue concentrations of [^{14}C]deoxyglucose. These autoradiographs are pictorial representations of the relative rates of glucose consumption in the various structures of the brain. Therefore, even without quantitation the autoradiographs can be used to map functionally related neural structures and pathways simultaneously in the entire brain."

The new method offers extraordinary advantages over existing methods of studying brain function, and Dr. Sokoloff and his colleagues are already exploring a number of fascinating problems: for example, (1) how does morphine affect brain function? At what sites does morphine alter activity? (2) LSD induces a psychosis-like state in human subjects; in animals, what does LSD do to brain function? What brain structures show altered activity after administration of LSD? (3) An infusion of epinephrine produces a feeling of anxiety, a kind of artificial or pseudo anxiety. What brain areas are affected? What parts of the brain mediate the experience of anxiety? The number of problems important to mental health which can be studied by this method is almost endless. It has elicited wide interest from research laboratories in many countries, a number of which have sent staff members to Dr. Sokoloff's Laboratory to take part in his research and learn the new method.

It is clear that the value of Dr. Sokoloff's discoveries is very high. It is equally clear that the perfection of his method in 1975 casts a new light on much of the research leading up to it during the past 15 years. Work that may have seemed fruitless at the time can now be seen to have borne very elegant fruit, and that happening is fortunate for us all and enormously pleasing to those responsible. I bring these matters up because the subject of research evaluation, particularly in the intramural programs of NIH and NIMH, has been almost a central theme this year, and I want to make some comments about it. It is useful to introduce the subject with an instance, so typical of basic science, where true evaluation can only be made after many years have passed.

It is not entirely clear why "evaluation" and "accountability" came to the fore this year as much as they seem to have. It is true that a year ago I forecast more attention to these issues, saying that "There appears to be increasing sentiment among both administration and scientific staff of the IRP for more critical and systematic review and evaluation of the research of laboratories and individuals." I added that, "I hope that the Board [of Scientific Counselors], bolstered by ad hoc consultants as needed, can handle a good deal of this problem." Among internal reasons for the increased interest is the continuing struggle in intramural research to live within a shrinking budget, with the consequent necessity for making more and harder decisions about research quality. In addition, partly for the same reason, members of the scientific staff, as represented by the Assembly of Scientists, have urged more systematic use of research review in making program decisions. Externally there are evidences of pressure from some points in the academic community for subjecting intramural research to a process akin to the "peer review" used for extramural applications. In government generally the theme of "accountability" is played more often, and the President's Panel on Biomedical and Behavioral Research, which was appointed early in the year, has raised questions specifically about the scrutiny to which intramural research programs are subjected.

I want to summarize what we've done and where we are, rather than take space to editorialize at length. In the first place I felt that intramural programs could not be treated like grant programs, with pre-review of all projects by external review panels, and that there was no point in debating that issue. Secondly, it has always been clear that our responsibility is to carry out research of high quality, and to utilize review and other methods which will insure that quality. Third, we had an instrument, the Board of Scientific Counselors, which was developed to aid us in evaluation of our programs, and our first obligation, and opportunity, was to present to the Board any suggestions or recommendations for changes in procedure. We therefore planned to engage the Board in a discussion of evaluation issues during the March 28-29, 1975, meeting, and I prepared a statement outlining the matters at issue. After summarizing the history of the Intramural Research Program and of the Board of Scientific Counselors, the development of the evaluation issue, and the reasons for its consideration, I concluded as follows:

> "This paper has been dealing with two questions which need to be kept separate, though they are related. They are (1) what changes, if any, should be made in our procedures for review and evaluation of the Intramural Research Program, and (2) what role in that process does the Board of Scientific Counselors feel it can and should play? In this section I would like to list and briefly comment on several options that relate to one or both of these questions.

"A. Duplicate in the Intramural Program the review system used in the research grants program. I mention this only to discard it, since I think it entirely impractical. It would mean that all intramural research "projects" would have to be reviewed in advance by panels of outside experts. It would be impossible, and I think wrong even if possible, to get enough interested and competent outsiders to review all of the projects of a full-time staff the size of ours, let alone the size of the NIH's. Furthermore it would, I think, be impossible to administer a research program like ours if decisions on support had to be made by outside panels. The resources we deal with are budgeted positions, space, and money. They cannot be awarded and withheld with the flexible abandon of dollars in research grants, nor can decisions on projects be made without regard for the impact of those decisions on the careers of the scientists in a full-time, largely tenured service. Finally, and most important, a pre-review of "projects" would so change the intramural program that it could no longer provide scientists with what is the unique virtue of intramural research--the opportunity for real continuity of effort over time.

"B. Continue the present system unchanged. In briefest outline the present system consists of post-review of each laboratory every 3 or 4 years, by the Board of Scientific Counselors assisted by ad hoc consultants, with the evaluation based primarily on (1) the reviewer's prior knowledge of the research, (2) perusal of the CV, bibliography, and presentation abstract of each scientist reviewed, and (3) oral presentation of the scientist's work at a Board meeting.

"C. Expand the present system by providing more information to reviewers. (The recommendation from the Assembly really fits here, even though the language of their report in places sounds more elaborate than the modifications I have in mind.) Perhaps the most useful kind of information not now routinely available to members of the Board and consultants is that gained through visits to the laboratories under review. We could provide as much opportunity for such "project site visits" as reviewers are able and willing to accept. Our staff would welcome the chance to discuss their work this informally. Could any system of periodic visits be worked out that would be satisfactory to the Board? Several possibilities come to mind: (1) Board members from the discipline or disciplines to be reviewed (others also if they wished), and the selected consultants, could spend a day visiting the labs under review just before the Board meeting. (2) The same kind of visit could be done at another time, if more convenient. (3) Each Board member could select one or two laboratories of his choice and plan, through visits convenient to him, to develop familiarity with the staff and their work. Such a special relationship could continue during the Board member's tenure, or a shorter period. Such visiting would usually turn into a kind of consultation, with benefits to the laboratory, as well as providing background to assist in evaluation.

"Finally, Board members and consultants could be given copies of a laboratory's more recent or more important publications sometime before

> a Board meeting. The catch here too is the time of Board members. It would be easy to supply these reprints; the question is, would Board members be able to read them?
>
> "These are the possibilities as they appear to us. Other and better suggestions may come from the discussion at the Board meeting. Though questions about program evaluation are less crucial than those having to do with program design and administration, the former do need careful study and thoughtful answers. We will welcome the comments and suggestions of Board members and consultants."

Discussion lasted most of Saturday morning, but was inconclusive so far as future courses of action were concerned. No member of the Board, or other participant, argued that we should attempt to duplicate the extramural peer review system in the IRP. All wanted the Board reviews to be as helpful as possible, but there was not sufficient time to work out a practical set of new or revised procedures that would be more expert and thorough, and yet be within the time and effort limits of the Board members. Subsequent to the meeting the new Chairman, Dr. Eleanor Sheldon, suggested that we decide, prior to the October 1975 meeting, on the agenda for the March 1976 meeting, and that the plan for review in March be decided on in October. It would thus be possible to tailor-make the review process to the subjects for review; perhaps in this way a satisfactory general plan of action could gradually be evolved. The suggestion is a good one, and we will propose it to the Board in October, along with a proposed agenda for the March meeting.

The issue of review came up again on May 15, 1975, by way of a request from the Staff Director of the President's Panel on Biomedical and Behavioral Research. In a memorandum Dr. Richard Louttit asked that the Panel be provided with answers to four questions about each intramural research program in NIH and ADAMHA. The fourth question had to do with "mechanisms for continuing review of research programs to assure continued high quality," and since my response dealt primarily with that issue, I am attaching my statement as an appendix to this report. In the statement I described our internal and external review procedures, but attempted to make clear also that in my judgment reviews are not the most important factor in developing a first-class intramural research program.

Since I have said some things about the Board of Scientific Counselors let me include here the other notes I wished to record about the Board. The members during the year were Dr. Robert Stubblefield, Chairman, and Drs. Walle Nauta, Albert Lehninger, Eleanor Sheldon, and Eleanor Gibson. Dr. David Hamburg, whose term on the Board begins July 1, 1975, served throughout this year as a special consultant and participated in both Board meetings. To our great regret, Dr. Lehninger felt compelled to resign from the Board in March 1975 because of the press of duties as a member of the President's Panel, and because of possible conflict in those two roles.

The terms of Drs. Stubblefield and Nauta expired on June 30. Both have been of great value to the Board and we are most grateful for their interest, concern, and hard work.

For the coming year Dr. Eleanor Sheldon has consented to serve as Chairman. New members of the Board will be Dr. Hamburg; Dr. Frederic Worden, Executive Director, Neurosciences Research Program, MIT; Dr. William Doty, Professor, Center for Brain Research, University of Rochester School of Medicine; and Dr. Nelson Goldberg, Professor of Pharmacology, University of Minnesota Medical School. During the year our request to enlarge the Board to seven members so that we could add a representative of pharmacology to our usual psychiatry, psychology, sociology, biochemistry, and neurophysiology was approved by the Office of the Secretary. We go into the new year with only six members, however, because the vacancy left by Dr. Lehninger's resignation has not yet been filled.

The Board met twice during the year. On October 25-26, 1974, it reviewed the Laboratories of Neurochemistry and of General and Comparative Biochemistry. In addition to the regular members of the Board and David Hamburg, we invited as special consultants in biochemistry Dr. John M. Buchanan, Professor of Biochemistry at MIT; Dr. Jordi Folch-Pi, Director of Scientific Research at McLean Hospital (and a former member of the Board for eight years); and Dr. Jarvis E. Seegmiller, Chairman of the Department of Medicine, University of California at San Diego. On March 28-29, 1975, the Board reviewed the research on affective disorders being carried out in the Adult Psychiatry Branch and in the Laboratories of Clinical Science and Clinical Psychopharmacology. Special consultants, in addition to Dr. Hamburg, were Dr. Eugene Bliss, Chairman of the Board last year, and Dr. Bernard J. Carroll of the Mental Health Research Institute, University of Michigan. Drs. Daniel X. Freedman and Lawrence Kolb were invited but were unable to attend. Also in March, as indicated above, we engaged the Board in a discussion of review and evaluation of intramural research.

Organizationally the Intramural Program finally returned this year to its former status as a legitimate "program," as a result of a favorable decision in the Office of the Secretary on our appeal for reversal of the earlier nomenclatural decision. We are, in other words, a line operating organization composed of three Divisions, rather than an "Office of Research Coordination" with no authority over the elements of the NIMH intramural research enterprise. From December 1973 to March 14, 1975, the IRP and its Director were in hierarchical limbo, on the organizational charts (and *de jure*) as research coordinators, but continuing to operate *de facto* as a line organization. The final decision of the Assistant Secretary for Administration and Management extended to all health research institutes the privilege of having an intramural program as a line operating level. So ends what has to be viewed as a tempest in a nomenclatural teapot, a tempest which blew up as an almost routine exercise of standard prudent administrative principles in a department so very large that the author of the rule was many echelons away from those on whose operations the rule would eventually bear.

An unusual feature of our past three years, not previously mentioned in these reports, has been the availability to the IRP of a fluid fellowship fund of $150,000 awarded by the Grant Foundation. We asked for the funds in order to be able to bring on more post-doctorate or pre-doctorate fellows than our limited position ceiling and budget would permit. The three-year period ends

March 31, 1976. We have used the funds primarily for post-doctorate candidates, and to date have awarded five full one-year stipends and eight supplemental or short-term stipends. Almost all of the funds have gone to our basic science laboratories, where positions are extremely tight, and where additional post-doctoral fellows can be accommodated. We have been able to meet most of the needs of the clinical laboratories from our regular budget. The Grant Foundation awards have supplemented our regular research training opportunities in important ways, and have given us the prized flexibility in timing and selection which only private funds can provide. We are most grateful to Douglas Bond, President, and Philip Sapir, Director of the Grant Foundation, for their interest in and faith in the Intramural Program.

An innovation this year has been the delegation to Scientific Directors of de facto authority to approve promotions of scientific support staff members from GS-9 to GS-11. This is an issue which has concerned many members of our staff, and members of the EEO Council have been active in pushing for change. Prior to this year all such promotion recommendations have needed to be approved by the Deputy Director for Science, NIH, after review and favorable recommendation by the assembled Scientific Directors. To assist in making decisions on such promotions most Scientific Directors have appointed standing Promotion Committees, as have we. Our committee consists of Dr. David Jacobowitz, Chairman, and Mr. Garrett Bagley, Mrs. Elena Battenberg, Mrs. Gladys Deibler, Dr. Charles Halverson, Dr. Norton Neff, and Dr. Stanley Rapoport. The Associate EEO Officer and the IRP Personnel Officer serve as ex-officio members. The Committee has worked hard and well the few months they have been in office, and there is every reason to believe that their efforts will be a real contribution.

The Intramural Equal Employment Opportunity Council continues to function well under the leadership of Mr. Erskine Davis, Associate EEO Officer and Mr. Harry Thompson, Coordinator, contributing useful advice and counsel to help us create a climate in which equal employment opportunity becomes a fact of life. One of its major accomplishments this year was a revision of the IRP Affirmative Action Plan.

Finally it is necessary to acknowledge with regret the departure, by retirement, resignation, or change of duty, of a number of our senior staff members during the year. Several of them had been with us more than 20 years. They have all helped in their own ways to make the Intramural Program the success it has been, and we are profoundly grateful to them for the dedication of a fair portion of their lives to the NIMH research mission. Those who have left are:

Dr. Richard Q. Bell, Chief of the Child Research Branch, retired to become Professor of Psychology at the University of Virginia.

Dr. Philippe Cardon, Laboratory of Clinical Science, transferred to the NIH as Associate Director of the Clinical Center.

Dr. Thomas Chase, Chief of the Section on Experimental Therapeutics, Laboratory of Clinical Science, transferred to the NIH as Scientific Director, NINCDS.

Dr. Edward Jerome, Laboratory of Psychology and Psychopathology, retired.

Dr. Morris Rosenberg, Chief, Section on Social Structure, Laboratory of Socio-Environmental Studies, resigned to become Professor of Sociology, State University of New York at Buffalo.

Dr. Robert Ryder, Chief, Section on Family Development, Child Research Branch, resigned to become Professor and Chairman of the Department of Child Development and Family Relationships, University of Connecticut.

Dr. Wilson Taylor, Laboratory of Clinical Psychopharmacology, retired.

This has been a lean year for honorary and other awards, apparently. My Special Assistant, Ms. Marguerite Hamburger, tells me there was only one, and I report it here because it was in my view a reflection of the esteem with which the NIMH Intramural Program is viewed in the Department and beyond. It was to the Director of Intramural Research, by the National Civil Service League, in the Career Service category. My thanks go to those who approved the award, but even more to those who wrote the presentation. I had to tell the crowd assembled in the State Department's Madison Room that it seemed sinful to receive an award for doing what has almost always been fun.

APPENDIX June 17, 1975

INFORMATION REGARDING THE NIMH INTRAMURAL RESEARCH PROGRAM

Prepared for the President's Biomedical Research Panel

John C. Eberhart, Ph.D.
Director of Intramural Research, NIMH

This statement will discuss in turn each of the four issues raised by Dr. Louttit's memorandum of May 15.

1. Difficulties in the retention of top staff, especially as losses may result from salary ceilings or from other resource problems.

   Loss of top staff is a problem. Numbers are not great but those who are attracted away tend to be the most gifted and thus represent a major loss to the Intramural Research Program. (For example, Dr. Floyd Bloom, Director, Division of Special Mental Health Research and Chief, Laboratory of Neuropharmacology, and one of our most creative young scientists, is leaving in the fall to take a position at the Salk Institute.) Reasons for these departures vary and are sometimes complex, but there is evidence that salary ceilings and declining resources are the major factors.

2. Adequacy of manpower. The Panel understands that some losses of support staff may be hampering research efforts.

   Position strength in the Intramural Research Program is down 21% since 1968. Research support and clinical care support positions (e.g. nurses), which are supplied by the National Institutes of Health, are also down. This decline has made it extremely difficult to carry on research and, particularly, to mount new programs. Clinical research, where there have been many promising new leads, is heavily dependent on manpower both for clinical care and for research. It is clearly moving at a slower pace than it would were more manpower available. A case in point is a series of studies on hyperactivity in children. This work is deemed so important that projects are being started with only one or two subjects at a time. Thus, accumulation of sufficient data on which to base conclusions will be extended over a long period of time.

   There are at present insufficient numbers of research support positions at NIH (e.g., procurement clerks, voucher payment clerks, laboratory and animal technicians, glassware handlers, building custodians). These shortages have a deleterious effect on all research in that they make research services much more difficult to obtain and result in costly delays and reduced productivity.

3. Flow of talent into the intramural programs. Has the end of the doctor-draft decreased the number of top quality young physicians who have sought research training at ADAMHA? Is the recruitment of young scientists a problem?

The end of the doctor-draft for a time threatened the very existence of good clinical research; and it has certainly decreased substantially the number of top quality young physicians seeking research training at ADAMHA. Clinical investigators, foreseeing the problem, embarked upon a nation-wide, intensive recruitment effort with some good effect. Additionally, new regulations which permit higher salaries (variable incentive pay) for those M.D.'s who are fully trained in an appropriate medical specialty and who are willing to join the USPHS Commissioned Corps, have helped to ensure adequate staffing.

There appear to be no major problems in recruiting young Ph.D. scientists; the only constraint is the lack of position ceilings.

4. Mechanisms for continuing review of research programs to assure continued high quality. The Panel would appreciate an explanation of the procedures used, peer review or other, by each Institute to assure that each of its intramural research programs is of high quality.

The most visible reviews of intramural research programs are those given outside the laboratory, such as those by the Board of Scientific Counselors; the most important reviews, however, are probably those made on a day-to-day basis within the laboratory. As necessary as are external reviews, they do not substitute for close and careful scrutiny of emerging new research at all stages by informed colleagues and superiors. Such internal review is facilitated by the nature of intramural research at NIH-ADAMHA. Both forms of review are described below.

Before outlining specific review procedures I want to touch on a more general question, which might be phrased as, "How do you insure continued high quality in your intramural research program?" The answer would involve not only review mechanisms but also all the other issues of program operation that determine its character and account for its long-term success. I want to suggest some answers to the question, "What must go into intramural research before review mechanisms have any function to perform?" Without trying to write a manual of laboratory operations, let me just note the following points:

1. Intramural research is conducted by a full-time staff as a direct operation of the agency, and is supported by appropriated funds.

2. Highly competent scientific leadership is essential, and is the most important element.

3. The most important decisions are in the selection of scientists for the staff and their differential encouragement by promotion and the provision of resources. No supervision and no reviews, however

critical, will substitute for the integrity and competence of the individual investigator. Program leaders must foster these qualities. Meticulous consideration of tenure appointments is vital to the long-term health of the staff, and the delayed tenure provisions of the NIH Staff Fellowship program and of the Reserve Commissioned Officer program are very important assets. Fewer than 7 percent of these temporary postdoctoral appointees have been given tenure in the past 8 years.

4. Intramural research is a career service, and the management of scientific careers is important both for the program and the scientists. The enterprise must be conducted in such a way that good scientists are encouraged to stay and less good ones to leave. There must be as much concern for career productivity as for current productivity. Some young scientists, like young prize fighters, need to be brought along slowly with challenges appropriate to their skills. Other young scientists need to be given their heads and encouraged to tackle big problems early.

5. The persistent efforts of the manager of a research laboratory must be to get the best research possible out of the scientists on his staff. It is more important for him to encourage research than to stop it. He must avoid the temptation, in the interest of preventing bad research, to erect barriers that stultify the research effort, limit imagination, restrict exploratory efforts to known methods and familiar problems and otherwise encourage "safe" research that satisfies standard criteria at the expense of originality.

6. The training functions of an intramural program are almost as important as its research functions. The immense potential of these programs for training a new generation of scientists must be utilized in the public interest; and the intramural programs have to depend on the scientists they train to fill leadership positions because senior scientists cannot be hired from outside the Federal Government at present Government salaries.

With these features understood, how is research reviewed in the NIMH Intramural Program?

Internal Review

Though laboratories are apt to vary in a number of ways, most will make use of fairly standard techniques in the initiation of research projects and in the early and continuing review of progress. The point of these techniques is to bring to bear the wisdom and judgment of the laboratory chief and his senior colleagues on proposed new research, and on the conduct of projects which have been approved. The assumption is that the laboratory chief, whose approval is generally required before start of a project, has acceptably high standards of what constitutes good research, that other senior scientists approach this level of expert judgment, and that when they are satisfied the project will have passed a severe test. It would be self-serving to argue here the superiority of our laboratory chiefs and of their scientific judgment, but it may not be amiss to point out that they have been selected for their eminence in science, and that their task is defined as primarily scientific leadership rather than "management" of a laboratory. Their first obligations are to stimulate good research and discourage bad research, and they are chosen first of all because we believe them to know which is which. Self-serving though it may be, I must say that their standing in the world of science, their service as officers of scientific societies and as editors of scientific journals, their experience on boards of research review either in universities or independent laboratories, or in NIH-ADAMHA initial review groups, equip them well for this role.

It is true of course, that no scientist knows all even of his own subdiscipline, and laboratory chiefs like other scientists are aware of the limits of their knowledge. All from time to time will invite in to serve as reviewers or advisors outside experts whose skill and experience can supplement that available within the laboratory. Often when a proposed problem involves techniques with which laboratory members are not sufficiently familiar, collaboration will be sought with another scientist who is an expert in the technique. In one laboratory bimonthly visits of leading scientists are scheduled in such a way that the visitor not only gives a seminar on his own work, but spends most of the day visiting and discussing with laboratory scientists the research they have underway, later to express his views on these matters to the laboratory chief.

In smaller laboratories, the chief is often able to stay in almost daily touch with all research in the laboratory, and such close and direct relationships with the scientists and their research is standard operating procedure for a number of laboratory chiefs in this program. He thus is able to exercise a decisive voice in the starting of projects, and to influence the future course as experimental results indicate. Such supervision is closer with more junior staff. In each laboratory there are usually one or more senior scientists besides the laboratory chief, and with scientific maturity they gain increasing autonomy.

No laboratory relies wholly on person to person interaction for review and evaluation of research. In all laboratories there are one to three formal research conferences each week, normally attended by all scientists in the laboratory. The conferences provide for discussion and criticism of proposed new research, and reports on current research, all of which are subject to the criticism of laboratory scientists. Time is also given to reviews of current published research, reports on scientific meetings, and consideration of possible new directions of the laboratory program.

In larger laboratories there ordinarily are meetings of research sections where the same functions are carried on.

Most laboratory chiefs have periodic research conferences with each scientist in the laboratory for in-depth evaluation of his activities. Normally such meetings include the section chief also if there is one. At issue in such conferences is the investigator's progress in his research, consideration of whether results so far warrant continuation, or increased resources, or possibly termination, and whether other research problems, not yet started, may have higher priority than the current investigation.

Finally, all laboratory chiefs review all manuscripts prepared for publication by members of their staffs. Their approval is required before an article can be cleared for publication, unless the author elects to publish without identifying the laboratory or the NIMH. (No instance of this has yet occurred.) Laboratory chiefs often send manuscripts back for rewriting, and sometimes for new data or new experiments. In the end, of course all manuscripts are reviewed by journal editors and their referees before publication.

External Review

Outside the laboratory review continues in several ways. Annually, the Director of Intramural Research, in formal session with Division Directors participating, reviews with each laboratory chief the work of all of the scientists in his laboratory. Some section chiefs also participate in these meetings, which are aimed at assessing progress and deciding which investigators should be considered for promotion in the coming year. The annual progress reports prepared by each investigator are useful in these reviews. It is probably fair to say that these reviews tend to be more severely critical than those by the Board of Scientific Counselors.

For certain larger projects, especially those which require additional funds or staff, ad hoc committees may be appointed to conduct special reviews. Their advice is utilized in deciding whether to approve the project and to allocate the new resources needed, or to disapprove it.

Research projects which involve the collection of data by interview or questionnaire are submitted for review by a Technical Review Committee composed of scientists and statisticians from the staff and outside. This committee, appointed by agreement with DHEW and OMB to carry out the purposes of the Federal Reports Act, reviews carefully all such studies for scientific merit, for statistical adequacy, and for public policy considerations, and may recommend approval, rejection, or modification. All protocols reviewed by the committee are also reviewed by PHS, DHEW, and OMB. Committee members are able, their standards for approval are high, and their approval is generally accepted by OMB as proof of technical adequacy.

Clinical laboratories and branches, and behavioral science laboratories, all do a good part of their research with human subjects, either patients or normal child or adult subjects. All studies from the Division of Clinical and Behavioral Research involving human subjects must be formally written up and reviewed by the NIMH Clinical Research Review Committee, a group of 13 members chaired by Dr. Robert A. Cohen, Deputy Director of the Intramural Program. A list of the members is attached. Four are from outside the NIMH. This group meets once or twice a month as needed, and usually reviews 4 or 5 proposals at each meeting. The committee considers the scientific merit of the proposal, the safety of the subjects, and all ethical considerations including procedures for securing informed consent. It may recommend approval, disapproval, or modification.

Three kinds of studies reviewed by this committee must be further reviewed by other groups. Studies involving normal control subjects, and non-therapeutic studies done on patients, must be reviewed by the Research Committee of the Clinical Center Medical Board, prior to final review and decision by the Deputy Director for Science, NIH. Studies involving the use of experimental drugs for therapeutic purposes are reviewed by from one to three committees in FDA after the NIMH-NIH reviews.

Research involving human subjects in the Division of Special Mental Health Research, located at St. Elizabeths Hospital, is reviewed within the Division and then goes for additional review to the Research Review Committee of the Hospital. On the basis of that Committee's recommendation the Superintendent of the Hospital makes his decision about final approval of the project.

The most formal public review of intramural research is given by the NIMH Board of Scientific Counselors. This group, with a membership of 7, is composed of leaders in American biomedical and behavioral science chosen from universities and other nongovernmental institutions. Since the members of the Board normally come from a wide range of disciplines--currently they represent neurophysiology, pharmacology, psychology, psychiatry, and sociology--it frequently happens that only one member of the Board will be familiar enough with a laboratory's work to judge it. To strengthen this review we began two years ago to invite in two to four *ad hoc* consultants each meeting from the research areas being reviewed to sit with the Board and participate fully in the review and formulation of the report. This has worked well and will be continued.

The Board meets twice yearly, for 1 1/2 to 2 days at a time, to review the research of one or more laboratories. The agendas are so structured that all 14 NIMH laboratories are reviewed by the Board at least every four years. Usually two laboratories are reviewed at each meeting, with each senior scientist and some junior scientists reporting on their work. The Board hears all presentations, questions each speaker, and at the end of the meeting in executive session reviews the work of all scientists for the Director of Intramural Research, the Intramural Research Program Division Directors, and the Institute Director when he can make it. After the meeting the Board prepares a written evaluation of the work for the information of program, division, and laboratory chiefs, and for transmittal of relevant portions to the scientists involved. Attached is the review of the Laboratories of General and Comparative Biochemistry and of Neurochemistry from the October 1974 meeting, the latest available. The report of the March 1975 meeting is not yet finished.

The role of the Board of Scientific Counselors in review and evaluation of NIMH intramural research is now under study. At the March 1975 meeting, the Board discussed its review functions on the basis of the attached statement on "Review and Evaluation of NIMH Intramural Research." The members endorsed their current mode of functioning as valuable to the program and consistent in scope with their responsibilities to their own institutions. They expressed interest in the possibility of taking additional time to visit the laboratories under review, and to read significant reprints and manuscripts, but left these issues open for further consideration at future meetings.

The Board's reviews, criticisms, commendations, and recommendations have been valuable to the program, both standing on their own and as supplements to the judgments of program leaders and the evaluations which come directly and indirectly from the scientific community. Decisions about increases or decreases in allocations of space, supporting staff, and money to scientists are guided by these evaluations.

Annual Report of the
Director, Division of Clinical and Behavioral Research
National Institute of Mental Health

July 1, 1974 - June 30, 1975

Robert A. Cohen, M.D., Ph.D.

As the years pass one acquires a certain sense of perspective which places our activities and achievements in contexts which would otherwise not be readily perceived. One becomes aware of how the spirit of the times determines both the opportunities we are afforded and the institutions which provide and support these opportunities.

As I write this annual report, the program of this Division is nearing the end of its twenty-second year of actual operation in the Clinical Center, having been established only six months earlier. The Director of the National Institute of Arthritis, Metabolism and Digestive Diseases and I are the only members of the first Medical Board still active on this campus. Each of the other original Institutes has had at least three Clinical Directors in this period.

How can one convey some sense of what happened in those years? The whereabouts of former staff is one indication. Of the Chiefs who established the six laboratories and branches which comprised the original program, one retired and remains as the first Scientist Emeritus appointed at the National Institutes of Health, three went as Professors and Chairmen of Departments of Psychiatry and Behavioral Sciences to Johns Hopkins and Stanford Universities, one as Professor and Director of an Institute at the University of California at Berkeley, one as Distinguished Professor of Behavioral Science at Wayne University. These men were already recognized as leading investigators in their respective fields when they came to this program. A second generation whose scientific reputations are based on their achievements while working in this program have gone on to Professorships and, in several instances to Chairmanships, at Harvard, Yale, Columbia, Cornell, Boston, Chicago, Baylor, University of California at Davis, University of California at Los Angeles, Johns Hopkins, Utah, Maryland, University of Illinois, University of North Carolina, University of Massachusetts, University of Virginia, Rochester, Duke and others. Each discipline represented in the program is included in these appointments: Psychiatry, Social Psychology, Developmental Psychology, Experimental Psychology, Sociology, Biochemistry and Pharmacology. The list of awards granted by national and international scientific organizations and foundations is equally impressive. Of present staff, all of the laboratory and branch chiefs, and fully 75% of the section chiefs have been strongly recruited for professorships at leading universities. The content and nature of their work is fully described in the reports of the individual laboratories and branches on later pages.

It need hardly be stated that despite these achievements our program did not march steadily forward to undiluted glory. The results of several research projects have been meaningless or banal. With the advantage of hindsight we have wondered how we even came to undertake them. All investigators try to "unscrew the inscrutable" in Adolf Meyer's pithy phrase, and a few have spent years without turning their particular screws more than a degree or two. In some instances we may have mistaken dogged unimaginativeness for thoughtful persistence. However, there is some merit in permitting the investigator himself to decide when his study should be discontinued. Paul Ehrlich tried 605 compounds before he found salvarsan - number 606 - the first effective treatment for syphilis. These are the vicissitudes of the lives of investigators and administrators in science. Our professional careers are, or should be, spent in attempts to maximize creative and minimize non-creative efforts. In the early years of the program that is precisely what happened. Our proposals were not given unquestioning approval, but it was generally accepted that our principal task was to do research, and for that we were given very good support. This support was not given in recognition of achievements already made but in recognition of the fact that this was the way in which achievements would be made. And they were made. In my view, they were made not only because the men and women who came to this institution were gifted; there are equally gifted individuals the wide world over. I believe that the several Institutes of NIH succeeded in developing an environment that facilitated the creativity of gifted individuals. An essential aspect of that environment was the remarkable degree of freedom afforded us in the selection of problems on which we chose to work and of the strategies we employed in their study. It hardly need be emphasized that by "freedom" I do not mean "license." A steadily modified and, hopefully, improving system of reviews were developed by the scientists and program directors to insure so far as possible the most thoughtful utilization of our energies and resources, and the most rigorous evaluation of our findings.

As the years have gone by the relative abundance of money for research (and for the travel, attendance at meetings and consultation which are an important part of modern research life) has been seriously restricted. This is unfortunate and I wish it were not so. But everyone is aware that our country has serious financial problems. I do not believe that basic biomedical and behavioral research can or should remain a calm oasis richly supplied with nutrients while the rest of the nation's enterprises endure grave deprivation. Our fortunes must rise and fall together. As the reports on the following pages show, we continue to do worthwhile research, and even to move into important new areas when such ventures appear promising.

However, accompanying this constriction in the research budget, serious questions have been raised about the research management and choice of problems in the intramural programs. There are now some restrictions on our freedom to utilize our resources in the ways we may deem most productive. Our selection of advisers and consultants must be reviewed and approved at points distant from the program level. With increasing frequency, decisions which affect our operations are made in conferences in which we do not participate. There is talk of an imposed external review of our plans and undertakings.

The creation of the President's Biomedical Research Panel and the suggestion that all intramural research programs utilize peer review procedures such as are employed in the extramural programs imply that our enterprises would be notably strengthened by changes in the management techniques which helped bring them to their present level. There is a difference in the atmosphere - not insupportable, not entirely unreasonable but nevertheless mildly oppressive and, in my view, one which has serious implications for an environment which is intended for the support of creative investigations.

Looking back, it seems to me that we misinterpreted the freedom we enjoyed in the early days of the Clinical Center. Many of us felt that the Federal Government had decided that the search for the causes, cure and prevention of diseases was its responsibility, that it had embarked therefore on a broad program of biomedical and behavioral research, and that the intramural NIH programs were given the responsibility and opportunity to focus sharply on basic problems in their respective areas of concentration. For a number of years we answered the questions put to us, explaining the possible ultimate relevance of our studies, pointing to this or that practical application of our findings, proudly displaying our Nobel and lesser prizes - always secure in the belief that we were doing what was expected of us. I wonder now whether we failed to recognize early dissatisfaction with the way we defined our mission and our objectives, dissatisfaction which culminated in the firing of two successive Directors of NIH after the imposition of lesser administrative controls failed to produce the desired results.

The review of a research institution's policies and practices by a group of serious and distinguished scientists cannot fail to be of benefit. But perhaps the most important contribution the Biomedical Research Panel might make would be a clear definition of the mission of NIH, mutually understood and agreed upon by the staff here and by the Congress which gives voice to the wishes and hopes of the people. I hope that it will be decided that the primary task of the Clinical Center is to carry out basic research since I believe there should be at least one such center in this country. If it should develop that the present and projected size of the NIH and NIMH intramural programs reflect an unexpressed expectation of early practical applications, I would prefer to see these programs shrink in size to the level the Congress feels it can reasonably devote to an Institute which devotes itself to fundamental studies of life processes. I do not mean by this that practical applications would not spring from this Institute. But attempts to evoke them by repeated and impatient inquiry, by demanding justifications of every proposal, and by evaluating a journey into the unknown as if it were a gigantic engineering project only serve to impair the effectiveness of such programs.

In closing, I wish to draw special attention to several organizational changes in this Division. Dr. Marian Yarrow has undertaken the responsibility of organizing a new program bringing together in the Laboratory of Developmental Psychology many of the staff and resources of the old Child Research Branch; Dr. Carolyn Waxler from the Laboratory of Socio-Environmental Studies, and Drs. Jacob Gewirtz and Albert Caron from the Laboratory of Psychology. Dr. David Rosenthal has abolished all of the old sections in the Laboratory of Psychology with the exception of the Section on Neuropsychology. A change in the goals and orientation of the group is reflected in its new name: Laboratory of Psychology and Psychopathology. The Adult Psychiatry Branch has begun work on its long-projected studies of hyperactive children and of children who have a manic-depressive parent. All these and other developments are fully described in the reports from the laboratory and branch chiefs.

Annual Report of the
Acting Director, Division of Special Mental Health Research
National Institute of Mental Health
July 1, 1974 - June 30, 1975
Floyd E. Bloom, M.D.

The scientific activities and productivity of this Division remained at the same extremely high levels demonstrated in the past, despite the presence of numerous disruptions, and the continual battles against staffing turnovers, research support levels which restrict the size of our staff and funding levels which have not permitted inflationary increases to be absorbed. In addition to these constant and more or less expected problems of everyday federal scientific life, the past year has seen some signs that our agreements with NIAAA (to operate the research laboratory space in the Animal House Annex) and with SEH (to achieve more prompt review of scientific protocols for clinical research and to achieve better mutual understanding of the needs and assets of SEH and SMR) may provide a more stable future on the local front. On the other hand, my resignation to become effective October 1975, will again produce the wholly reasonable tensions which the Division experienced during the period after Dr. Salmoiraghi's departure was announced.

During the fiscal year 1975, Dr. Wyatt returned from Stanford University to a laboratory which was already in full swing under the acting leadership of Dr. J. C. Gillin. Since Dr. Wyatt's return, the Laboratory has continued to be extremely active in devising new attacks on the biochemical pathology which may underlie schizophrenia, emphasizing the enzymatic differences which characterize cells of these patients and the possible behavioral effects of the compounds which could arise _in vivo_ as the result of the possible abnormal enzymic activity. A notable development which characterizes the scientific activities of this Division is the close collaborative effort between SMR/P and SMR/C, particularly with respect to the development and application of new chemical methodologies, such as mass spectormetry, monoamine oxidase characterization, and analysis of the turnover of transmitter substances in fluid or cell samples of extremely small size.

Dr. Costa's Laboratory has again continued to exhibit a strong leadership role in psychopharmacology at the fundamental level. Now that scientific groups throughout the world are attempting to follow his lead in instituting programs based upon mass spectormetric analysis of small brain regions "fixed" _in vivo_ by powerful microwave irradiation beams; SMR/P has forged ahead again by demonstrating for the first time that the methods of High Pressure Liquid Chromatography can be utilized for the most sensitive assay yet available of serotonin synthesis. Using this method, they have gathered more direct evidence that p-Cl-amphetamine and fenfluramine are able to produce direct decreases in serotonin synthese in specific cell group. Furthermore, Dr. Costa and Dr. Guidotti and their younger associates have demonstrated that it is possible to develop methods which will elucidate the molecular sequences by which sympathetic neurons and brain cells are activated or regulated by neurotransmitters and which express themselves through the intermediation of cyclic nucleotides and the transfer of phosphate groups onto (or off of) specific proteins.

In the SMR/N, notable scientific achievements have also been obtained by further application of the methods developed here for the analysis of drug and synaptic transmitter action at the molecular, cellular and behavioral levels of analysis. As in the previous several years, the emphasis of this laboratory has been to combine cytochemical methods with those of single cell electrophysiology to gain a most complete picture of the neurons in various regions of the CNS to which catecholamine fibers project. In this past year, these methods have been extended at one end of the spectrum to cells raised under fully viable conditions in culture, to neonatal brain material transplanted into the anterior chamber of the eye where it survives and becomes sympathetically reinnervated, and to genetically mutant mice whose cerebella lack specific target neurons and synaptic interconnections. At the other end of the spectrum the methods have been applied to behavioral model systems in the rat hippocampus and the squirrel monkey cerebral cortex revealing the likelihood that a major significant regulatory function over certain types of learned responses may be mediated by the norepinephrine pathways.

(All these studies are described in somewhat more detail by each of the individual laboratory narrative summaries which follow.)

Dr. Weil-Malherbe, approaching mandatory retirement at the middle of fiscal year 1976 has continued his scholarly investigation of the ammonia metabolizing sequences of brain slices *in vitro*, and has in addition accomplished a very interesting set of studies on the effects of adenylate deaminase activation by these metabolic products.

However, we cannot rest here, satisfied with things as they are. Dr. Costa has indicated in his narrative report that some personnel and budgetary increases or reallocations would be required for the maintenance of his multi-leveled, multi-directional attacks. These requested changes are caused partly by the very aggressive manner by which Dr. Costa fully utilizes every inch of space which has been assigned to his unit, requiring from each of his people the high levels of self-fulfillment he demands of himself. The request is also in part due to the changes in administrative utilization of Visiting Scientist appointments which have previously been permitted within other-than-permanent positions.

It seems to me that while Dr. Costa's request for substitution of four permanent positions for four other-than-permanent positions could be accomplished within the Division in fiscal year 1976 with the anticipated vacating of several permanent positions within SMR/D and SMR/N, it is not now fully clear that making the substitutions from these resources would in fact be the best use of these resources. Also to be considered is the question of whether these laboratory staffs will soon have to be restituted within the NIH spatial domain of NIMH if the SEH interaction deteriorates while awaiting clearcut word of Congressional intent regarding the NIMH future at SEH, and the question of what will become of SMR/N or the leadership role expected of SMR/D. My opinion is that the decisions must be made logically from the top down, rather than pre-emptively from the bottom up. Therefore, I hope that we can within the very near term future begin to discuss and act vigorously to determine the future of the Division here and the role of the SMR/D; following that, the status and future of the units which will remain in SMR/N, and following those decisions, the possible redistribution to other labs within SMR of the personnel resources which will become available here.

In closing, I wish to state my very sincere appreciation to all those scientists and supporting staff who have given so generously of their time to contribute to our Division's efforts over the past and preceding years, and who have been so tolerant of my divided efforts to provide some continuation of the administrative care of the Division. This record of your past achievements is a notable one which has established the Division and its Laboratories as an international leader among biological psychiatric research throughout the world. Having established the methods and the application of the methods and being faced with possible future problems, I realize that it will be extremely difficult to maintain the same significant flow of activities even with our past momentum. Nevertheless, I fully expect that regardless of an incumbent in the SMR/D, the laboratories of this Division will continue to demonstrate their expert scientific talent and do so with the same activity and energy as in the past. With this plea for continued full steam ahead while the administrative readjustments are being implemented, I bid you all a sincere farewell.

ANNUAL REPORT OF THE ADULT PSYCHIATRY BRANCH
National Institute of Mental Health
July 1, 1974 - June 30, 1975

William E. Bunney, Jr., M.D.

The Adult Psychiatry Branch has continued in the process of transition since Dr. Lyman Wynne resigned as Chief in 1971. On 14 November, 1973, Dr. William E. Bunney, Jr., became the new Chief of the Branch. The past year has been involved with reorganization of the APB, with the development of new research protocols in the areas of genetics, pharmacology, biochemistry, and physiology, and with the recruitment of appropriate staff. Over 40 new pharmacological protocols have been initiated by the Branch Chief and the professional staff, passed by the Clinical Research Committee and by the FDA Review Committee. The basic concepts and hypotheses underlying these protocols will be reviewed briefly below.

The reorganization of the APB was completed on 6/19/74 and now consists of four clinical programs and four support programs. Clinical programs include clinical research units involved with the study of acute schizophrenia and the study of affective illness. In addition to these, a program has been developed in drug abuse and one in childhood mental illness. The four support programs are concerned with studies of psychogenetics, biochemistry, sleep, and perception and cognition.

The two clinical sections are functioning well. The first, the Section on Neuropsychopharmacology, is concerned with genetic, pharmacological, biochemical and psychological studies of acute schizophrenia, and will be headed, as of July 1, 1975, by Dr. van Kammen as Unit Chief. This unit is primarily concerned, at the present time, with the study of the dopamine hypothesis of schizophrenia. The drugs which activate or reverse schizophrenic symptomatology specifically affect dopamine receptors. A number of protocols to test this hypothesis are currently in use on this ward. Dr. Carpenter has completed a creative and energetic year writing up the previous research data derived from this clinical unit.

The second Section, entitled the Section on Psychobiology, administers a clinical ward which is concerned with affective illness. The Unit Chief is Dr. Robert Post. This unit has been concerned with a variety of programs including the study of patients who trigger the switch process between depression and mania, studies of calcium metabolism, and new pharmacological agents which are specifically norepinephrine or dopamine agonists.

The Unit on Childhood Mental Illness has experienced a difficult year in attempting to establish a collaborative research program with the Laboratory of Clinical Science on Ward 2-West. Most of the obstacles have now been overcome in terms of protocol approval, recruitment of staff, and training of professional and para-professional personnel. In April, 1975, the first children were admitted to the unit. Dr. Brown has headed up the collaborative effort for the APB and Dr. Ebert for the LCS. The initial goal of this program is to study hyperactive children and the mode of action of methylphenidate. In addition to this project, a collaborative effort has been initiated among Dr. Brown, Dr. Gershon's Unit on Genetics, and Dr. Goodwin's Section in the LCS to study the clinical state of the children of patients who have been admitted to the NIMH in the last ten years with affective illness.

The Unit on Studies of Drug Abuse is headed by Dr. Arnold Andersen, who has developed protocols to study the ability of pharmacological agents to block the euphoria produced by drugs of abuse. A number of these protocols are ready for use in the clinical units. The past year of preparation has laid the groundwork for admitting the first drug abuser during the summer of 1975.

Dr. E. Gershon has been the chief investigator in the Section on Psychogenetics. He also has developed a broad program which is intensively collecting clinical data at the present time concerning genetic factors in affective illness and is searching for genetic markers in this disease state. Within this Section, Dr. Herbert Weingartner has worked in the area of learning and memory and has developed collaboration with most of the units in the Clinical Center NIMH Intramural Program.

The Section on Biochemistry has provided minimal biochemical support for the units, since no laboratory space was available to the APB during the first 12 months that Dr. Bunney has been Chief. The only technician working in the Section on Biochemistry has been Miss K. Black. Dr. Tallman, a biochemist, has been recruited and has spent the last year learning techniques in the laboratory of Dr. Axelrod. It is anticipated that the initial laboratory space for the APB will be available during the summer of 1975 and that biochemists will be recruited to provide biochemical support for the research units.

During the past year, two new units were added to the APB. The first was the Unit on Perceptual and Cognitive Studies. This Unit was previously shared jointly with the Psychology Branch. The full transfer of this Unit to the APB appears to be working well and the productivity of the program is continuing.

Dr. Frederick Snyder left the NIH, and a part of his Section on Sleep Studies was also transferred to the APB. Dr. C. Gillin and Dr. R. Wyatt are helping to coordinate the scientific efforts of this Sleep Unit. It is anticipated that the Sleep Unit will provide a valuable service for all five of the clinical wards in the NIMH Intramural Research Program.

In addition to the above programs, a collaborative effort with the Cancer Institute has been initiated to study the role of psychological stresses in triggering malignant melanoma. In the future, it is anticipated that a study will be developed to investigate the role of psychological stress in breast and lung cancers.

One area of major new focus in the Branch involves the study of receptor function in man in psychopathological conditions and during the administration of pharmacological agents. A paper entitled, "Systematic Strategies for the

Study of Receptor Function in Man" was written and published by Drs. Bunney and Murphy and develops strategies and a new theory concerning the etiology of manic-depressive illnesses. Protocols are now being written and studies are being initiated on Ward 3-West to test these hypotheses. It is felt that the last decade has been devoted to understanding the presynaptic neuron and its role in mental illness and biology, and that the next decade may well focus on understanding the interaction between the presynaptic and postsynaptic neurons and the postsynaptic and perhaps presynaptic receptor. Hopefully, this will lead to a further understanding of psychopathological processes in man.

During the past year, a maximum effort has been made to support those investigators who are leaving, such as Drs. Carpenter and McGlashan, in order to facilitate the analysis and publication of data which has been collected over the past few years. In most instances, this has involved secretarial and research assistance support. It is felt that this approach has tended to decrease the individual stress and trauma of the change in the focus of the program and provides for a full exploitation of the previous research efforts.

Intensive effort has been made to coordinate all of the APB programs and new initiatives with the work of other research efforts in the Intramural Program. The Clinical Investigations Coordinating Committee has continued to provide a useful forum for sharing information and a method for activating collaborative efforts.

Honors and Awards:

President of the Psychiatric Research Society, 1974-1975. Appointed to the Lithium Task Force, American Psychiatric Association, 1974-1975. Appointed Chairman of the Program Committee, American College of Neuropsychopharmacology, 1975.

Invited lectures included: Mt. Sinai School of Medicine; Columbia University; Maimonides Medical College; American Society for Human Genetics; World Psychiatric Association Society Meetings on Biological Psychiatry.

Publications:

Murphy, D.L., Biegel, A., Weingartner, H. and Bunney, W.E.,Jr.: The Quantitation of Manic Behavior. In Pichot, P. (Ed.): Psychological Measurements in Psychopharmacology. Modern Problems of Pharmacopsychiatry. Paris, Karger, Basel, 1974, Vol. 7, pp 203-220.

Murphy D.L., Goodwin, F.K. and Bunney, W.E., Jr.: The Psychobiology of Mania. In Arieti, S. (Ed.): American Handbook of Psychiatry. New York/London, Basic Books, Inc. Vol. 6, in press.

Murphy, D.L., Baker, M., Goodwin, F.K., Miller, H., Kotin, J. and Bunney, W. E., Jr.: L-tryptophan in affective disorders: indoleamine changes and differential clinical effects. Psychopharmacologia 34: 11-20, 1974.

Bunney, W.E., Jr., and Murphy, D.L.: Strategies for the Systematic Study of Neurotransmitter Receptor Function in Man. In Usdin, E. and Bunney, W.E., Jr. (Eds.): Pre- and Post-Synaptic Receptors. New York, Marcel Dekker, Inc., 1975, pp. 283-312.

Ng, L.K.Y., Szara, S. and Bunney, W.E., Jr.: On understanding and treating narcotic dependence: a neuropsychopharmacological perspective. Br. J. Addic. In press.

Gershon, E.S., Bunney, W.E., Jr., Leckman, J.F., Van Eerdewegh, M. and DeBauche, B.A.: The inheritance of affective disorders: a review of data and of hypotheses. Behav. Genet. In press.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Irwin J. Kopin, M.D., Chief
July 1, 1974 through June 30, 1975

Introduction

The Laboratory of Clinical Science comprises six sections and the Office of the Chief. Three of the sections (Pharmacology, Medicine, and Histopharmacology) are engaged mainly in fundamental research while three (Psychiatry, Clinical Psychopharmacology and Experimental Therapeutics) are concerned mainly with clinical investigation. During the year, Dr. Michael Ebert, formerly Chief, Unit on Clinical Pharmacology in the Office of the Chief, LCS, has been Acting Chief of the Section on Experimental Therapeutics, replacing Dr. Thomas Chase, who had been appointed Scientific Director of NINDS. There has been a gradual change in the research of this Section, with increased emphasis on metabolic abnormalities, behavioral disorders in children, and clinical pharmacology. The Office of the Chief contains three units: The Unit on Clinical Biochemistry and the Unit on Analytical Biochemistry continue to function in support of the clinical research units, as well as independently, developing new methods required for the research. A new, third unit, on Pharmacological Applications of Mass Spectroscopy has been established with Dr. Sanford Markey as Chief. This was required because of the large investment of the Laboratory in these highly sophisticated instruments and the need for some one person to coordinate, facilitate, and optimize their use for the various projects requiring the sensitivity, specificity and ability to detect non-radioactive "heavy" isotopes provided by mass spectroscopy.

The research conducted in the Laboratory of Clinical Science is directed towards integrating fundamental knowledge obtained about how nerves and brain function with clinical investigations of the abnormalities which may be of importance in the etiology of neuro-psychiatric disorders. The programs of basic research are aimed at elucidating the metabolic, neurophysiologic, genetic, and hormonal processes which influence the growth, development and function of neurones and their synapses and how pharmacologic or other interventions alter these processes. The clinical investigations strive to determine the biological bases of abnormalities of brain function, to describe attendant biochemical changes, and to determine the distribution, metabolism, and biological effects of drugs which influence the clinical state of patients. In addition to independent programs of research, there is a good deal of cooperation and collaboration between the various units and sections within the laboratory and with other branches of the Intramural Program of the NIMH and the other institutes.

Section on Pharmacology
Julius Axelrod, Ph.D., Chief

Investigations in the Section on Pharmacology have continued to examine the mechanisms for control of the formation and utilization of biogenic amines and their actions on receptors. Enzymatic methylation reactions are the subject of investigations as well as tools in microenzymatic assays of the amines. Drs. Diliberto and Viveros have continued studies on a methanol-forming enzyme, protein carboxymethylase, which was found to be highly localized in rat brain and concentrated in the synaptosomal fraction. The enzyme is also highly localized in the cytosol of the adrenal medulla and an acceptor protein for this enzyme appears to be concentrated in the membrane of the adrenal medullary chromaffin granules. Preliminary evidence for a new methyltransferase for membrane lipids also has been found.

Several micromethods for the assay of biogenic amines and enzymes in specific brain nuclei have been developed.

A new method for tryptophan hydroxylase has been developed by Drs. Kizer, Zivin, Saavedra and Brownstein. Dr. Saavedra has described a new sensitive method for 5-hydroxytryptophan decarboxylase and Drs. Tallman, Saavedra and Axelrod, a new method for tyrosine hydroxylase. Glutamic acid decarboxylase has been assayed in brain nuclei by Dr. Brownstein in collaboration with Dr. Tappaz (Section on Medicine). These methods and the development of the brain microdissection techniques (Drs. Palkovits, Brownstein, Saavedra and Kizer) made it possible to map about 130 nuclei of the brain for their content of biogenic amines and synthetic enzymes and to study the effects of various endocrine manipulations. Tryptophan hydroxylase has been found to be highly localized in serotonergic nerve cell bodies and axons.

Drs. Saavedra and Zivin have mapped the location of dopamine-β-hydroxylase and tyrosine hydroxylase in the limbic area. Repeated stress/results marked changes in serotonin content of the raphe nuclei (Drs. Saavedra, Palkovits, Brownstein, and Kopin) and lesions in the locus coeruleus affects serotonin metabolism in raphe (Drs. Saavedra, Jacobowitz, Brownstein and Grobecker). The distribution of serotonergic terminals examined after raphe lesions showed that cell bodies in the raphe project axons into the limbic area as well as the hypothalamus (Drs. Palkovits, Jacobowitz, Kizer, Saavedra and Brownstein). Combined neurochemical and anatomical studies show that lesions in the locus coeruleus produce degeneration of fibers and disappearance of norepinephrine in cortical and hypothalamic areas (Drs. Palkovits, Brownstein and Saavedra). A similar study on the effects of lesions in the substantia nigra was carried out (Drs. Kizer, Palkovits and Brownstein). A cholinergic pathway originating in the habenula and ending in the interpeduncular nucleus was found (Drs. Czaba (Budapest), Brownstein and Palkovits). Effects of PNMT inhibitors and experimental hypertension on PNMT in discrete areas of the brain are being examined (Drs. Saavedra, Grobecker and Axelrod).

Dr. Brownstein is studying the effects of hypothalamic deafferentiation on levels of biogenic amines and hypothalamic hormones inside and outside of the hypothalamic island. The distribution of GAD (glutamine acid decarboxylase, a marker for GABA) has been examined in 65 brain nuclei (Drs. Brownstein, Tappaz and Palkovits). A substantial number of GABA nerve terminals seem to be present in hypothalamic nuclei. Somatostatin (a peptide that inhibits the release of growth hormone) is present in other brain areas in addition to the hypothalamus. Somatostatin, LHRF, and TRF are being examined (Drs. Brownstein and Kizer).

A method for the direct measurement of the β-adrenergic receptor has been developed by Drs. Zatz, Romero, Kebabian and Axelrod. It has been found that the number of available β-adrenergic receptors and the sensitivity of adenylate-cyclase are reduced by prior β-adrenergic stimulation.

Dr. Kebabian, in collaboration with Drs. Carpenter and Shain (AFRRI) have found that venom from Russel's viper contains a heat labile, non-dialyzable component which selectively antagonizes the dopamine-induced increase in adenylate cyclase activity from rat caudate nucleus, the pineal β-adrenergic receptor in response to isoproterenol and the adrenal ACTH sensitive adenylate cyclase. Thus, the venom would seem to function as an "uncoupler" of the receptor-adenylate cyclase complex. Drs. Romero, Zatz and Axelrod showed that stimulation of the β-adrenergic receptor enhances the generation of cyclic AMP which, in turn, acts at two sites to give increased enzyme activity. One site involves the stimulation of RNA synthesis (transcription) and the other involves the stimulation of protein synthesis (translation). Furthermore, there is an endogenous diurnal rhythm in the synthesis of messenger RNA related to N-acetyltransferase activity.

Section on Medicine
Irwin J. Kopin, M.D.

The research projects in the Section on Medicine continue to focus on the neurotransmitter substances released from nerve endings. Their aim is to illucidate mechanisms of control of synthesis, storage, release and action of biogenic amines and to determine their role in normal physiological responses, in pathological states, and in mediating the effect of drugs.

Methods for sensitive and specific assay of catecholamines in plasma of animals and Man have been developed and applied to studies of physiologic effects, several disorders of the sympathetic nervous system, and the action of drugs. In animals, Drs. Reid and Kopin found that drugs which block sympathetic ganglia markedly depress plasma catecholamine levels while receptor blocking agents elevate levels of these amines. Dr. Roizen found that exposure to cold elevates levels of plasma catecholamines, while warming animals or administration of a variety of anesthetic agents depresses catecholamine levels. With Dr. Thoa, Dr. Roizen showed that anesthetic agents appear to have a direct action on sympathetic nerve endings, depressing stimulation-induced release of norepinephrine. In spite of extreme and prolonged changes in plasma catecholamine levels, dopamine-β-hydroxylase, which had been thought to be an index of sympathetic neuronal activity, does not change. Spontaneously hypertensive rats have higher plasma catecholamine levels than appropriate controls, but they are both relatively low compared to normal Wistar rats.

Drs. Ziegler and Lake have examined plasma catecholamine responses to postural change and isometric hand exercise in normal subjects and in patients with neurologic or psychiatric disorders, before and during administration of psychoactive drugs. Plasma catecholamine levels increase with age, respond to such relatively minor stresses as insertion of the venepunctur needle, and under resting conditions are stable in given individuals over a period of weeks. The levels increase with assuming the erect position and with exercise. Levels are low and do not increase in patients with the Shy-Drager syndrome and in Familial Dysautonomia. While hypertensive patients appear to have normal levels of plasma catecholamines, the levels are depressed when reserpine is administered and when hydrochlorothiazide succeeds in lowering the pressure. They are elevated when adrenergic blocking agents are administered. Dopamine-β-hydroxylase levels vary widely, are stable for years in the same individuals, but appear to be increased in patients with the dominant form of torsion dystonia. These patients also have increased resting levels of catecholamines which show the usual increase with procedures which normally elevate levels of these amines. In patients who develop an increase in blood pressure, DBH levels appear to decline.

Dr. Stephen Kizer has produced antibodies in rabbits to Thyroid Releasing Factor (TRF) and Luteinizing Hormone Releasing Factor (LHRF) and developed radioimmunoassay procedures for these factors. In collaboration with investigators in the Section on Pharmacology he has localized the site of these factors in the hypothalamus nuclei and mediam eminence. Dopamine appears to be present at sites appropriate for control of TRF while the distribution of choline acetylase parallel that of LHRF.

Dr. Zivin has studied the distribution of amines in the spinal cord and found that a lesion results in an immediate and striking decrease in norepinephrine and serotonin content of the gray matter. Dr. Tappaz, with Dr. Brownstein, is examining the distribution of glutamic acid decarboxylase, the enzyme responsible for formation of GABA, in the central nervous system and they are examining the effects of hypothalamic deafferentation.

Dr. Antonia Liuzzi found that treatment of newborn animals with NGF results in increased PNMT in the sympathetic ganglia and adrenal medulla. Affects of NGF are also apparent in the pineal and parts of the brain. Methods for producing specific chemical lesions in brain are being explored.

Dr. Charles Popper has developed a means of quantifying behavioral effects of different doses of amphetamine in rats of different sizes. He has found that the responses to low doses of amphetamine may produce effects opposite those of higher doses; this may be related to the sedative effect of amphetamines in hyperactive children.

Section on Histopharmacology

David M. Jacobowitz, Ph.D.

The research done in the Section on Histopharmacology has focused on the aminergic neuronal systems in the central and peripheral nervous system. With Dr. Palkovits, a complete brain atlas indicating the localization of catecholaminergic and acetylcholinesterase-containing neurons in various nuclei of the brain of the rat has been completed. A similar atlas of the localization of catecholaminergic neurons and serotonergic cell bodies in the monkey brain is currently being prepared in collaboration with Dr. Mac Lean. The concentrations of norepinephrine and dopamine in discrete nuclei or brain subdivisions are being determined in the rhesus monkey brain using an enzymatic-isotopic assay.

A modification of the micropunch technique for removal of nuclei and other small regions of the rat brain has been developed whereby fresh unfrozen discrete regions can be removed by the use of a vibratome. This will be useful for isolation of synaptosomal membranes, in tissue culture and for electron microscopy.

Studies in collaboration with Drs. Ziegler and Thomas have shown that after intravenous injection of the antibody to dopamine-β-hydroxylase (ADBH), demonstrable by an immunofluorescent method, adrenergic neurons and adrenal chromaffin cells are capable of taking up the antibody. The antibody persists in sympathetic ganglion cell bodies, nerve terminals and in the adrenal medulla up to ten days after injection. ADBH injected into the anterior chamber of the eye resulted in fluorescence of DBH-containing nerves in the iris a few hours after injection. These results show that ADBH can bind to intracellular structures in living animals and that after it is taken up at nerve terminals can be transported by an antidromic flow to the nerve cell body.

A histochemical study of catecholamine-fluorescent nerves in an isolated perfused rat brain was undertaken in collaboration with Dr. Sloviter (Univ. of Pennsylvania). After 40 minutes of perfusion, the quality of the varicose terminal fluorescence and the cell bodies of the noradrenergic, dopaminergic and serotonergic neurons was excellent. The isolated perfused brain preparation can provide information about the influence of drugs on neurotransmitters without interference or ambiguity caused by other organs.

In collaborative studies with investigators in the Section on Medicine, the effects of acute stress on NE and DA concentration and repeated stress on tyrosine hydroxylase activity were measured in individual hypothalamic nuclei and other rat brain regions. NE and DA concentrations were reduced and TH activity increased selectively in the arcuate nucleus. These results suggest that the arcuate nucleus may be selectively involved in the stress response and support the hypothesis that catecholamines in the medial basal hypothalamus mediate certain of the neuroendocrine changes observed in stress. A chronic isolation stress (3 months) resulted in an increase in the NE concentration in the cingulate cortex and substantia nigra (zona compacta).

The dorsal noradrenergic bundle of the rat brain has been obtained using the micropunch technique, and NE, DA and DBH were assayed within this axonal tract. The ability to remove a segment of a noradrenergic axonal pathway in the brain will provide a useful model for the study of the synthesis and transport of amines and of enzymes and other specific proteins to their terminal synaptic regions in the brain.

The effect of two anesthetic agents, halothane and cyclopropane, on the catecholamine and serotonin concentration of 20 discrete areas of the rat brain were studied with Dr. Roizen. Norepinephrine concentration increased in the nucleus accumbens, caudate putamen, the anterior ventral nucleus of the thalamus, locus coeruleus and a central grey catecholamine area in close proximity to the dorsal raphe cell bodies. An increase in dopamine was observed in the nucleus accumbens and arcuate nucleus. Serotonin was observed to increase in the raphe dorsalis, substantia nigra and the nucleus amygdaloid centralis. The differential influence of drugs on some discrete aminergic neurons remains to be explained.

Section on Psychiatry
Frederick K. Goodwin, M.D.

Clinical investigations of affective illness carried out in the section include behavioral and biochemical studies. In collaboration with investigators on the 3-West Nursing Unit (APB), Dr. Wehr has found that sleep deprivation for one night produces dramatic but transient improvement in some depressed patients, particularly those with more endogenous features. Transient improvement with sleep deprivation tends to predict subsequent response to tricyclic antidepressant.

A scale to rate functional capacity, based on occupational therapy performance rated by Ms. Schreiber (Occupational Therapist) has been developed and has been shown to have high interrater reliability.

Increases in amine metabolites in cerebrospinal fluid after administration of probenecid to drug-free depressed patients is used to assess the rate of formation of these metabolites. A non-normal, but apparently bimodal distribution is evident for serotonin and norepinephrine metabolites after probenecid administration and clinical correlates of these subgroups are now under investigation. The effects of psychoactive drugs on CSF amine metabolites appear to be time-dependent so that a substantial increase in probenecid-induced accumulation of HVA in the CSF is apparent after two weeks of treatment with phenothiazines, but this effect is lost after five weeks, in spite of higher daily doses.

We have previously noted that 2-3 weeks of lithium therapy is associated with a decrease in the probenecid-induced accumulation of 5HIAA, but recent studies indicate that after 3-5 days of treatment with lithium in most patients there was either an increase or no significant change in 5HIAA accumulation. Significant differences in probenecid induced accumulation of 5HIAA and HVA have been found between lithium responders and non-responders, and in the reverse direction between tricyclic responders and non-responders.

In collaboration with Edna Gordon, VMA in the CSF has been found to be significantly lower in depressed patients compared to neurological controls, manics or acute schizophrenics, paralleling our previous finds with MHPG. A study of CSF amine metabolites in various character disorders has been carried out in collaboration with the National Naval Medical Center, and we have found that levels of HVA in CSF of individuals with impulse control problems and histories of violent outbursts is significantly higher than in individuals with character disorders not associated with these problems.

The urinary MHPG excretion in large number of medication-free depressed patients has been found by Dr. Beckman to be similar to that of normal controls. However, significant subgroup differences in MHPG were found within the depressed population, with bipolar patients excreting significantly less MHPG than those patients with unipolar histories or the control subjects. Within both the depressed population and the age-matched normal controls (also studied on the Ward) there was a significant positive correlation between systolic B.P. and urinary MHPG. A diurnal pattern for MHPG excretion has been observed which may differ in depressed patients and normal controls.

Studies on the effects of pain - stress on urinary MHPG have been carried out by Dr. Muscettola in normal controls and in patients, when depressed and after recovery, with parallel measures of urinary steroids and cortical evoked responses (done in collaboration with Dr. Monte Buchsbaum). Differences between patients and controls on these stress response measures appear to be significant.

Studies by Dr. Rubovits on the endocrine related potentiation of tricyclic response in established non-responders have been continued using T3, and the phenomenon of potentiation in some patients is now more clear. On the other hand, T3 does not appear to potentiate the acute antidepressant response to lithium. Dr. Gold has begun studies on the potentiation by estrogens of tricyclic response in females.

In cooperation with the 2-West Nursing Unit, the efficacy of pimozide in the treatment of manic psychosis is under investigation, and is being compared with chlorpromazine as a reference neuroleptic. Three patients have responded dramatically, while four have shown only a gradual and incomplete improvement.

Dr. Thomas Wehr has undertaken longitudinal studies of behavioral, physiological and biochemical variables in inpatients and outpatients who evidence relatively rapid mood cycles. The characterization of circadian and other rhythms, examined by spectral analysis, indicate that some bipolar patients show circadian desynchronization in temperature and other biological parameters so that the rhythm is no longer "locked" in phase with the environmental day-night cycle, but tends to drift in and out of phase. Studies on the basic drive rhythm of 90-minutes are underway, utilizing the collaborative assistance of Dr. Howard Hoffman of NICHD, who has developed some new statistical methods (complex demodulation).

The increase in outpatient follow-up activities has been one of the most important developments in the Section in the past year. Former patients are readmitted briefly after being free of major symptoms for at least six months. A battery of behavioral and biological studies is completed for comparison with the previous depression or the period just after recovery. Outpatient couples group therapy as an adjunctive psychotherapeutic treatment appears to result in a considerable enhancement of the prophylactic efficacy of lithium. The efficacy of lithium vs. lithium plus tricyclics in control unipolar and bipolar affective illness is also being studied.

In collaboration with the Adult Psychiatry Branch the children of patients with affective illness are being studied. Preliminary results indicate a higher than normally expected incidence of diagnosable depression in the children of our affectively ill patients.

Section on Clinical Neuropharmacology

Dennis L. Murphy, M.D.

In continuing studies of whether clinical phenomena may help predict responses to drugs, two subgroups of manic patients identified on the basis of behavioral differences quantitated on the Manic State Scale were found to respond differently to lithium carbonate treatment. Building upon our experience with the Manic State Scale, a multicomponent Affective Disorder Rating Scale has been constructed and examined for reliability and validity. High correlations with other scales for depression (the Beck, Hamilton and IMPS subscales) and with other specific scales for mania, psychosis and related psychopathology factors have been demonstrated, and this optically-scanned, computer scored scale is now available for routine clinical use.

Further studies of psychoactive drug effects on memory and learning (in conjunction with Dr. Weingartner) have delineated impaired memory function during physostigmine administration, which is contributed to markedly by state-dependent phenomena. Similarly, d- and l-amphetamine memory effects were particularly state-dependent and could only be demonstrated when they were correlated with the activating effects of the drug in different patients. In a series of longitudinal studies of cycling manic-depressive patients, carried out by Drs. Weingartner and Miller, the specific contribution of affect-related states to the deficits in memory and learning seen in non-drug-treated manic and depressive patients was defined.

Dr. von Kammen found that d- and l-amphetamine activating, euphoria and antidepressant responses in depressed patients were markedly attenuated by lithium carbonate pretreatment, a finding which has implications both for understanding lithium's actions in mania and for the possible treatment of amphetamine "addiction. Detailed clinical studies of other aspects of the amphetamine stereoisomers actions, including those on sleep, evoked responses, plasma enzymes and hormones, and drug blood levels have been accomplished with other NIMH-IR staff, and proved to be extremely useful in verifying many individual patient response characteristics.

In Laboratory-based studies, platelet monoamines activity was found to help predict responses to some psychoactive drugs, but the degree of MAO inhibition observed during treatment with the MAO-inhibiting anti-depressant phenelzine did not correlate with behavioral responses. Ms. Donnelly and Dr. Richelson have compared the characteristics of platelet MAO activity with the MAO-A and -B subtypes found in brain, sympathetic ganglia and cultured glial and neuroblastoma cells. Thus far the platelet MAO appears to be essentially identical with the MAO-B subtype in other tissues. In studies of human platelet amine storage and transport, Dr. Costa has used electron microprobe and other techniques to quantify relationships between calcium, phosphorous and serotonin stored in vesicles. Using the platelet to study reserpine effects, two separable actions of the drug on the vesicle membrane have been identified: (1) an initial uptake-inhibiting action at the vesicle membrane and (2) a separable serotonin-releasing effect.

Section on Experimental Therapeutics

Michael H. Ebert, M.D.

With the departure of the former Section Chief, Dr. Thomas Chase (who has moved to the National Institute of Neurological Diseases and Stroke (NINDS) as Scientific Director), Dr. Michael Ebert has been appointed Acting Chief of the Section. Since the Section on Experimental Therapeutics involves the operation of a clinical research unit (2-West), a transition period has been required. This has involved a collaborative effort by Drs. Ebert and Chase to gradually shift the responsibilities for patient care and to establish new clinical research projects on the Ward. The previous focus of the Section had been on the study of neurotransmitter function and experimental therapeutics in neurological disease and this is to be continued in the Neurology Institute. During the year, the 2-West clinical research unit has been converted from a neurological service to a metabolic and psychosomatic service. There has been a shift towards a psychiatric orientation to patient care and collection of psychometric data on patients. The unit will remain flexible to admit a diversity of clinical groups depending on research needs. To complement the other NIMH clinical research units, the categories of patients studied will include psychosomatic disease, inherited disorders of metabolism, neuropsychiatric disorders, and psychiatric disorders of childhood.

Since the dementia of Huntington's disease is as serious a deficit as the choreiform movements, psychometric studies of the dementia were undertaken with Dr. Herb Weingartner. The initial studies pursued this year demonstrated an abnormality of information processing in Huntington's dementia which appear to involve mainly retrieval rather than the storage of information. Retrieval seems to be impaired because of an inability to utilize and generate cues to access memory.

In the area of psychosomatic disease, patients with anorexia nervosa are being studied in collaboration with the Reproduction Research Branch of NICHD. A battery of neuroendocrine function tests and a variety of tests to evaluate central and peripheral catecholamine metabolism are being used to evaluate hypothalamic function in these patients from the point of view of endocrine regulation and neurotransmitter function. Considerable evidence from studies in animals implicates central noradrenergic and serotonergic systems in the regulation of hunger and satiation. The patients will undergo several therapeutic drug trials the efficacy of which will be compared with a behavioral modification program.

Studies of minimal brain dysfunction in children have begun as a joint effort of the Laboratory of Clinical Science and the Adult Psychiatry Branch. These studies include a comprehensive work-up of a series of children with this disorder including psychological testing with a focus on attention and memory storage, tests of catecholamine metabolism that are appropriate to do in children, psycholinguistic studies, and several therapeutic drug trials. The drug trial in progress concerns methylphenidate, a drug used in clinical practice to treat minimal brain dysfunction. The pharmacokinetics of methylphenidate will be studied in relation to clinical response by use of a gas chromatograph-mass spectrometric assay.

In collaboration with Dr. S. Harvey Mudd, a metabolic study of a 15-year old patient with sarcosinemia, a rare inherited disorder of amino acid metabolism demonstrated that on normal dietary intake, the patient was carrying out some methylneogenesis which was increased by subnormal labile methyl intake, and decreased by increases in methionine of choline intake. The results are compatible with a "threshold" hypothesis for methyl group economy in Man.

In collaboration with Dr. Robert Post, a primate model was used for continuous collection of cerebrospinal fluid from parts of the ventricular system or from the lumbar space. Such metabolic experiments in rhesus monkeys serves as a model for experiments in Man that utilize lumbar cerebrospinal fluid, blood, and/or urine.

With Drs. Kartzinel and Chase, the probenecid test has been modified so that 40 mg/kg of the drug is administered intravenously over one hour. Blood and cerebrospinal fluid levels of probenecid have been determined. When administered intravenously, the plasma levels and half-life of probenecid show little variance between patients and the half-life of probenecid is not affected by the administered dose, as has been claimed in the literature. The relation of acidic metabolites of dopamine and serotonin to cerebrospinal fluid levels of probenecid was determined for 12 hours after administration of probenecid. This modification may lead to a more accurate estimation of central dopamine and serotonin synthesis in Man.

An approach to central norepinephrine synthesis in Man was attempted by Drs. Ebert and Kopin. In this study ^{14}C-dopamine is administered to patients to physiologically label peripheral norepinephrine stores and the specific activity in urine of two metabolites of norepinephrine, 3-methoxy-4-hydroxy mandelic acid (VMA) and 3-methoxy-4-hydroxy phenylglycol (MHPG) are determined. The experiments indicate that there is selective labelling of norepinephrine in sites where VMA is preferentially formed. A substantial portion of the MHPG excreted, derives from a source which is unlabelled by the radioactive dopamine. This source probably includes the central nervous system, but other sources may also contribute to the MHPG.

Work continues on stable isotope strategies to study brain catecholamine metabolism in Man. Drs. Ebert, Havens, and Kopin have administered deuterated methionine to primates and to rats and demonstrated labelling by the deuterated methyl groups of methionine of methylated catecholamine metabolites appearing in brain tissue or cerebrospinal fluid. These turnover experiments in animals may be useful in developing a procedure which can be applied to patients to give quantitative turnover rates of central catecholamines.

Office of the Chief
Irwin J. Kopin, M.D.

As indicated above, the Office of the Chief contains three units which develop methods for assay of drugs and biogenic amines and their metabolites in biological fluids. These techniques are applied to studies in primates and in Man.

The Unit on Clinical Biochemistry under the charge of Mrs. Edna Gordon has previously developed methods to measure homovanillic acid (HVA), 3-methoxy-4-hydroxy-phenethylene glycol (MHPG) and vanillyl mandelic acid (VMA) in cerebrospinal fluid (CSF) by gas chromatography-mass fragmentography. These methods have been adapted to the analysis of blood and tissue. Analyses of other compounds, such as dihydroxyphenylacetic acid (DOPAC) and dihydroxy phenethylene glycol (DHPG), are also now possible so that studies of metabolism of dopamine (DA) and norepinephrine (NE) can be included in examination of these compounds.

Studies involving patients have been continued with the Sections on Psychiatry, LCS, and with the Adult Psychiatry Branch. Special emphasis has been on the study of VMA, the acid metabolite of NE. After probenecid treatment this metabolite has been shown to increase in human lumbar CSF.

In monkeys, studies being done in collaboration with the Section on Experimental Therapeutics, NCI, indicate that there is an active transport process which removes metabolites of DA and NE from brain and spinal cord. Blockage of this transport by probenecid appears confined to the choroid plexus while capillary removal of the compounds is insensitive to probenecid. These studies also showed that HVA in lumbar CSF is mainly the result of metabolism in brain while MHPG appear to be derived from the spinal cord rather than brain. These studies will help evaluate the results obtained from the measurement of these metabolites in human lumbar CSF.

In collaboration with the Section on Experimental Therapeutics of LCS, studies using HVA labelled with the stable isotope deuterium the turnover rate of HVA in plasma of monkeys and humans has been shown to be a few hours. MHPG appears to be the major metabolite of norepinephrine in saliva, but the origin of this compound has not yet been defined.

Dr. Robert Colburn, Chief, Unit on Analytical Biochemistry, has continued studies on the effects of calcium ionophores on release of norepinephrine from synaptosomes. He is now engaged in separation of the various metabolites of 2-bromo-α-ergocryptine and with Dr. Sanford Markey will determine the metabolic fate of this dopamine receptor-stimulating compound.

After the arrival of Dr. Sanford Markey in November 1974, a unit on Pharmacological Applications of Mass Spectrometry was formed. This unit has been actively engaged in the development of assays for several drugs and endogenous compounds using mass spectrometry, and in the continuation of the application of several previously developed assays. Of major concern has been the upgrading and modification of existing gas chromatography-mass spectrometry instrumentation for more reliable usage at high sensitivity. There has been a striking improvement in performance of gc-ms pumping systems, helium separators, data systems, and control electronics. A critical evaluation of instrument and recording capabilities has been made in order to standardize procedures developed on the different mass spectrometers in our laboratory.

Projects now in progress are the development of assays for bromocryptine, nortryptaline, amitryptaline, desipramine, and imipramine, drugs which are of clinical interest to programs in the research wards. Continued progress with the use of a cocaine-norcocaine assay to study the pharmacokinetics and metabolism of this abused drug and its active metabolite in monkeys has been made.

Synthesis of catecholamine metabolites with stable isotope labels has been undertaken (homovanillic acid, vanillylmandelic acid, 3-methoxy-4-hydroxyphenylglycol, dihydroxyphenylacetic acid, dopamine) in order to provide compounds suitable as internal standards for gc-ms.

Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

July 1, 1974 - June 30, 1975

Marian Radke Yarrow, Ph.D., Chief

The Laboratory of Developmental Psychology became a new Laboratory in the NIMH Intramural Program in March, 1975. The statement of Laboratory functions, the basis for its establishment, is broad: The research conducted by the Laboratory is on the development of cognitive, social, emotional, and adaptive behaviors, from infancy through adolescence, in normal and clinical populations. The research focus is on basic psychological processes underlying development and behavior.

This newly organized unit reflects an administrative reorganization of existing programs. Although Developmental Psychologists have had a long history at NIMH, they have worked within at least three different Laboratories or Branches. The reorganization brings them together. The Laboratory has thereby a built-in history in the NIMH careers, the research interests, and the accomplishments of its staff members. It is fortunate that NIMH has been able to draw and hold psychologists who are distinguished in the discipline and who are identified as significant contributors to knowledge in Developmental Psychology.

The Laboratory could have continued, though I think unwisely, with research life uninterrupted, but for a new name and a new Chief. Such a decision would not have used reorganization as an opportunity to assess current research activities and to make explicit program decisions and the bases for their choice. Planning of program directions has been undertaken deliberately slowly, taking into account (a) the evaluations by NIMH administrators and scientists of the quarter century of NIMH research contributions, (b) the current state of knowledge in Developmental Psychology, and (c) the roles and responsibilities of developmental research at this time, in this institution.

Our primary objective is sound basic knowledge. Our emphases will be on affective and social aspects of development and child behavior. The conceptualization and investigation of these areas in molar behavioral terms can and will lead to consideration of variables of biology as well as social macrostructural variables. We will give increasing emphasis to children beyond the early years. We see no necessary incompatibility between sound conceptual and theoretical basic research and the selection of areas or issues that have recognizable import in the lives of children, in contributing to understanding and treatment of their maladaptive behaviors, and in contributing to the realization of their potentialities.

Since this is a first summary, it may be well to be explicit concerning the model and philosophy by which the administration of the Laboratory is guided.

Because we are in a process of reorganization and of defining program emphases, the various Section structures that were brought to the Laboratory from other Branches and Laboratories have been abolished. Eventually, an intralaboratory structure of Sections may be re-established.

As general Laboratory policy, flexibility will be maintained in the use of resources of personnel and facilities, since research needs and inspirations are inconstant. In this way, investigators can be supported with expanding or contracting resources as research purposes and phases justify. Further, since research support staff have many generalist skills, some flexibility in their assignment will be maintained to promote a more efficient use of personnel at this level, and at the same time provide the personnel with greater breadth of experience and training.

The organization of research projects is the prerogative of the senior investigators. There is no contemplation of a single unified program for the entire Laboratory. Since there are common interests among investigators, and optimal opportunities for scientific exchange within the laboratory, it is hoped that collaborative research planning will become a major part of the endeavor. Individual investigator research is likely to continue to be preferred by some investigators, for some research problems.

The work of the Laboratory has been and will continue to be facilitated by the presence of guest scientists who have chosen to work for a year or two with investigators here, because of their research interests in given areas.

A new senior investigator and new post doctoral staff Fellows will join the Laboratory in the Fall of 1975. Their research interests and expertise should contribute to the realization of some of the projected research initiatives of the new Laboratory.

As a means of exposing our research to the review and the ideas of colleagues in the field, suitable members of the discipline will, from time to time, be invited to the Laboratory to consult and critique specific projects. These will be informal and internal reviews designed to keep research at the highest quality.

Description of Research Projects

There is no tidy way of grouping the 19 research projects in this year's report. There is the former Child Research Branch longitudinal study of early development which, though essentially longitudinal in objective, accounts for projects addressing cross-sectional as well as longitudinal issues. Infancy is an area of heavy research involvement: infant perception, cognition, and learning, and infancy as the baseline for later development. A unifying theoretical issue of caretaker and child reciprocal effects runs through a number of methodologically distinct projects. Some studies touch on an interface of biological and environmental contributions to behavior. Other studies deal with the affective-social domains of development. These groupings, though motley in level and conceptualization, will serve loosely to organize the individual research contributions.

Cross-stage analyses from the Longitudinal Study. The longitudinal study which was conducted from 1966 to 1973 by the former Child Research Branch, is now in the completion phase. The past year has been solely a year of analytic work. The design of the study was such that four chronological stages pertaining to early child development (preparental characteristics of mother and father, neonatal functioning, mother-infant interactions during the first year, and functioning at age 3) were investigated separately and in relation to one another. The fruits of this study to date are briefly described below.

In order to proceed on analyses dealing with longitudinal progressions, it has been necessary to deal first with data reduction, since at each of the developmental stages many dozens of variables and measures were obtained. Dr. Moss has had the primary responsibility for this extensive labor and for the planning of a comprehensive cross-stage integration of the preparental, neonatal, infancy, and early childhood assessments. Factor analytic methods have been used as summarizing procedures. Final sets of variables, expressed as Factor scores for each stage, have been constructed. With these, an overall analysis of longitudinal sequential relations will be made to determine what are identifiable preparental and congenital precursors or predictors of development in later infancy and early childhood. The general cross-stage factor analysis has been given lower priority in the schedule of research reporting, however, until the investigators, Drs. Moss, Halverson, Waldrop, and Yang, have first reported on the individual developmental stages and on specific hypothesis-directed cross-stage questions.

One completed longitudinal analysis is an investigation of the "inversion of intensity" hypothesis. This is a replication study. In the original study of a cohort of subjects preliminary to the main longitudinal study, Bell, Weller, and Waldrop (1971) found an unanticipated relation between newborn functioning and 2½-year-old characteristics. Lethargic behavioral responses and low autonomic functioning in newborns were related to higher activity levels, greater social participation, and more advanced speech development at the preschool level--in other words, an "inversion of intensity" of activity across developmental stages. It was deemed important to establish the reliability of these unusual findings, since, if the findings are reliable, they would suggest a need to re-evaluate neonatal behavior and reinterpret neonatal predictive signs. Therefore, using the main longitudinal cohort, Drs. Yang and Halverson carried out, as nearly as possible, a replication of the analyses pertaining to "inversion." On a measure-by-measure basis, the "inversion" relation was not replicated. Further, based on Factor scores, low basal neonatal activity and low neonatal reactivity to stimulation are unrelated to high social participation level at age 2½, and are negatively related in boys to impulsive, frenetic, high acitivity at age 2½. Thus, this particular domain of early "markers" remains in some confusion and doubt and more investigation is required. Further, the relations between distant developmental points, such as neonatal status and 2½-year-old status, whether reflecting continuity or discontinuity between the points, leave unanswered the learning-process questions. For example, what possibly distinctive parent-infant interactions are set in train by very high or very low infant activity intensity, leading thereby to distinctive child behavior patterning at subsequent ages? Data for this kind of exploration will be available in later cross-stage analyses.

Neonatal studies from the Longitudinal Study. Using the extensive behavioral and physiological measures of the newborns in the longitudinal sample, Dr. Yang has examined associations between neonatal status and two temporally antecedent conditions, the psychological state of the mother during pregnancy and maternal analgesic medication during labor and delivery. He has found that maternal anxiety during pregnancy is related to greater amounts of obstetric analgesic medication, and in turn, aspects of analgesic medication are related to newborn homeostatic efficiency (drugs given earlier in labor are associated with increased homeostatic efficiency; drugs given closer to birth, with slightly decreased homeostatic efficiency). Such evidence suggests the importance of early psychological preparation of the mother for child birth. Dr. Moss' analyses add another practical dimension to this relationship. He found more depressive tendencies during pregnancy among the women in the lower socio-economic sector of the sample, indicating particular preparatory needs in different socio-economic groups. Provision of information by obstetricians and childbirth education groups which sensitively addresses maternal fears during the prenatal period could contribute to more efficient management of delivery and optimal neonatal outcome.

Further work by Dr. Yang during this past year has involved the derivation of a dimensional (factor analytic) description of the neonate. Three stable-over-time factors (Reactivity-irritability, Maturity, and Reflexive and Discriminative Sucking) were identified. Dr. Yang's measures, in contrast to the widely used assessment scales of neonatal behavior which sample primarily the waking and social-interactive infant behaviors, appear to assess more directly the infant's congenital equipment. These measures might provide important extensions in standardized infant diagnostic work. Further, to investigate the theoretical question of how the infant by virtue of his equipment at birth determines the nature of the human responses made to him, a relatively pure "congenital" measure is needed as a baseline.

Infant-mother interactions from the Longitudinal Study. Analytic work on infant-mother interactions during the first year of life (Dr. Moss) has been concentrated on deriving Factor scores preparatory to within- and cross-stage analyses; these analyses are now in progress, but are not at a point at which findings can be reported. A subsample of mothers who had been studied with their first-borns gave birth to a second child. Dr. Moss gathered the same mother-infant interaction records on these second-borns, thereby obtaining the necessary data for an investigation of possible maternal care variations associated with children's birth order. Birth order effects continue to be reported in the psychological and psychiatric literature. Differing frequencies of first-borns and later-borns appear in various mental illness categories (schizophrenia, alcoholism) as well as on personality dimensions (conformity, achievement, intelligence). If such relations are reliable, what are the possible processes by which children in differing ordinal position develop differently? From his direct observational data, Dr. Moss has found some differences in rearing conditions. Compared with first-borns, second-borns, especially females, receive reliably less social attention and caretaking from the mother in the first year of life. If the second-born is a male who follows a first-born female, the second-born suffers no such decrement.

Early childhood from the Longitudinal Study. Dr. Halverson and Dr. Waldrop designed and carried out an extensive and intensive assessment of the 3-year-old child, including cognitive capabilities, affective characteristics, activity level, social behavior toward peers and adults, and coping with stress. They also reassessed the children at age 7½. These measures provide the predictive outcome points for the earlier developmental periods in the longitudinal analyses. They provide also the data for investigating behavioral organization at 3 years of age, and behavioral consistency over time. Two sets of findings from the ongoing analyses have particularly provocative implications. The first pertains to behavioral consistencies and continuities. Drs. Halverson and Waldrop found that on an impressive number of behavioral dimensions there is high correspondence across a range of situations at any one time period, and consistency from age 2½ to age 7½. Such consistency and continuity appeared in the child's level and ease of social participation, social competence, ability to cope with frustration and peer aggression, and relative amounts of positive and negative interaction with peers. Also, multiple indices of hyperactivity showed significant stability over the five-year-period. This configuration of intersituational and over-time consistencies in interactional and affective behaviors early in childhood suggests that the 2 to 3 year level may be a critical point for intervention in deviant patterns of social behavior.

In a second group of studies, Halverson and Waldrop report findings on children's hyperactive (disruptive, impulsive, uncontrolled) behavior. Their findings demonstrate the complex determinants of these maladaptive behaviors. From their data on the same children at 2½ and 7½ and from two large samples of elementary school children, ages 6 to 12, a stable, replicated set of relations appears; namely, the presence of multiple minor physical anomalies (such as epicanthus, adherant ear lobes, curved fifth finger, widely spaced eyes, etc., presumed to be due to embryological developmental irregularities) is related to problem behaviors in both sexes. Anomalies are consistently and strongly related to uncontrolled hyperactive behavior in boys. In girls the associated behavioral disturbances have a less consistent form, sometimes impulsivity, sometimes inhibited, uneasy social behavior.

The investigators interpret the association of multiple physical anomalies and "problem" behaviors not simply as constitutional predetermination of behavior disorder. Instead, the hypothesis is advanced that interaction of congenital and experiential factors lead to the expression of disordered behavior in early and middle childhood. Evidence of association between congenital anomalies and the expression of disordered behaviors occurs along with consistent evidence of association between the presence of multiple minor anomalies and certain stable social experiences, namely, higher frequencies of negative affect and negative evaluations from peers and teachers. Further, hyperactivity is reliably associated with failure in intellectual performance, which in turn, by inference, is associated with further negative feedback from the social environment. There is not only a dramatically higher incidence of hyperactivity in boys in clinical and nonclinical populations; there is also reliably different female adult caretaker treatment of high intensity activity from boys and girls. There is a substantial developmental literature showing that from the earliest years female caretakers respond more negatively and are more "set" to institute negative control of boys' high intensity behaviors than of girls.

One might conclude that biological and experiential influences are not readily singly accountable for molar behavioral manifestations, such as specific behavioral disorders. Biological processes are the medium, possibly the roots, of some kinds of behavioral malfunction, but they occur in social and cultural environmental contexts such that one determinant acts upon the other, limits, conditions, or even produces the other.

Research on infancy. The research with infants, conducted by Dr. Albert Caron, is concerned with perceptual and intellective capacities. It attempts to tap more complex processes than are usually measured in the simple sensory-motor assessments in infant clinical work. For this purpose, a major commitment of time and resources this year has been to developing procedures (for measuring habituation and discriminative conditioning) and sophisticated instrumentation and technology adapted to the very young infant. Several hundred infants have been observed over multiple sessions and conditions in this process. Habituation, it is assumed, involves information-processing functions beyond simple sensory registration, namely, perceptual coding, short-term memory, and matching of coded to stored information. Discriminative conditioning involves, in addition, the infant's ability to discriminate stimuli, associate stimuli with appropriate responses, and inhibit inappropriate responses--the earmarks of problem-solving behavior. Differences in the above behaviors may well be indicators of variable intellective functioning very early in life, and as such they have potential as standard diagnostic or infant screening devices.

With the procedural and technological development accomplished, research is now underway in three separate studies. Two pertain to basic and long-standing issues in perceptual development and learning: The first concerns infant sensitivity to the depth dimension, a question which has occupied a prominent place in nativist-empiricist controversies. On the one hand, it is argued that the infant can make immediate use of such visual cues as binocular parallax, motion parallax, and optical expansion to perceive a three-dimensional world, a position which assumes built-in detection of depth. On the other hand, it is maintained that although such visual cues are discriminable, depth perception is the product of learning to correlate them with the sensations accompanying reaching and locomoting in space (i.e., that the hand "teaches" the eye). Dr. Caron's research procedure deals with the infant's ability to see a cut-out shape (i.e., a square) as constant despite its being tilted toward or away from him and thereby being projected differently on the retina (i.e., as a trapezoid). To the extent that the infant is able to preserve constancy, he must somehow be taking the orientation of the object in the third dimension into account. A familiarization-novelty procedure is used. These data are now being collected.

A second issue of infant learning, addressed through the discriminative operant training procedures, concerns the opposing theoretical views regarding the necessity and sufficiency of differential reinforcement and differential responding in early perceptual learning. Here, too, further data collection is required before findings can be reported.

Further along in data collection is a collaborative project under Dr. Caron's direction that speaks directly to the diagnostic value of the habituation and conditioning procedures, here, the value in assessing the effects of medical procedures on the infant. In this study, associations are examined between the use of obstetrical medications (analgesics, anesthetics, tranquilizers) and differences in infant habituation.

Also involved in the development of basic procedures for the investigation of instrumental-learning in infants, are Dr. Gewirtz and Dr. Poulson. Procedural variations are being investigated with interest in determining the most effective strategies for facilitating the learning of the young infant. For example, is it ultimately more efficient for the infant to be learning many things simultaneously, or does he learn more readily if allowed to master one set of skills before learning additional skills? This research is early in its course.

Reciprocal adult-child influences. Over the years, research findings on relations between parental rearing behaviors and child behaviors have been interpreted as the effects of parent on child. Theoretical formulations and research designs and procedures have perpetuated this one-sided emphasis on the caretaker as the determinant. They have influenced practice as research findings have been translated into rearing guidance and recommendations for clinical and remedial procedures with children. This single directional formulation has persisted despite major theoretical papers which present a strong case for considering dyadic relations or two-way influences between adult and child. Recently, investigators have begun seriously to take up the issue. The first stage of shift in research perspective is reflected in studies designed to investigate child effects on adult; i.e., a reversed, but still mainly one-directional analysis. A number of NIMH staff members are among those who have addressed this issue, first in the theoretical work by Richard Bell (1970), and then in empirical studies which have provided substantial documentation of the child's contribution to the adult's behaviors toward the child, and hence, by inference, the adult's effects on the child. (Moss in naturalistic interactions between mothers and infants, 1970; Yarrow, Waxler, and Scott in a laboratory study involving older children and adult caretakers, 1972.) In a just completed observational study of mothers as behavior models in the learning of preverbal children, Drs. Waxler and Yarrow document the bidirectional influences at work. The child's imitation influences the mother's subsequent (reinforcing) behaviors toward the child. The mother's manner of modeling and her reinforcement of imitation, in turn, influence the child's imitative responding. The current work of Gewirtz brings into clear focus the issue of the infant's contribution to the caretaker's behavior. He employs the instrumental-conditioning paradigm in an experimental mother-infant interaction to ask: Will the infant's orienting response or vocalization which the mother believes her infant has made to her reinforce a response that she has been making toward the infant? Will this influence-process occur without the mother's awareness? Simulated infant responses following the expressive maternal behaviors functioned to reinforce (increase in frequency) the mother's expressive behavior toward her infant. Thus the infant trains his mother. These experiments provide strong demonstration of significant child contributions to the patterned interactions involved in rearing. Their occurrence so early in the life process is especially significant. The critical issue for further research, to which Dr. Gewirtz is now directing attention, is to devise procedures by which the ongoing simultaneous reciprocal influences of both actors upon one another can be assessed. Investigation of this reciprocal-influence process is extremely difficult, and certainly extremely relevant to understanding or modifying any learned, adaptive and maladaptive patterned interactions between individuals of any age or relationship.

Affective development. Although a significant part of experience is affective, the human sciences have made relatively small advances in understanding the origins and dynamics of affective states and their consequences for individual adaptation, well-being, or malfunction. Questions of affect, developmentally and cognitively considered, have been the subject of a series of studies by four investigators in the Laboratory, Drs. Waxler, King, Barrett, and Yarrow. The studies focus on the development of one- and two-year-old children's emerging sensitivities to affective events in their environment (anger, pain, fear, joy, sadness in the persons around them), and their developing sensitivities to the affective consequences of their own behaviors for others. Although responding was not without inconsistencies and individual variation, children were often finely attuned to and discriminating in response to others' affects. In the second year, for example, tension and anger between parents predictably brought unambiguous responses, such as crying, trying to escape or block out the scene, and comforting or punishing one of the parents. Changes in reaction to affective stimuli are traceable in day to day records over 9 months. For example, early in development, crying by a sad or injured person tends to elicit contagious crying. This later gives way to sober, worried regard. Around 1 year of age, comforting responses appear, often in remarkably sophisticated form. Analyses now in process address experiential factors associated with differences in children's sensitivities to affect.

A related line of investigation has focused on the affective behaviors expressed by the child to others. One question of particular interest was whether different kinds of compassionate behaviors tend to be highly interrelated or whether they tend to be empirically independent. Does the young child who tends to show concern for a crying peer also tend to share with him when he has the opportunity to do so? Is the child who shares his toys to a great extent likely to give physical assistance to an adult who has dropped some materials? Examining children, ages 3 to 7, in natural and experimental situations on several indices of "prosocial" behavior, only modest relationships among the various aiding behaviors were identified. All significant relationships identified were between sharing and comforting. Physical helping was not related to the other two behaviors. The data provided evidence that prosociality is not a unitary concept and suggested that to speak of prosocial behavior without specifying the nature of the response being studied, manipulated, or measured, could lead to theoretical confusions and to improper clinical inferences.

A second facet of the same study examined the compassionate behaviors of children in relation to their "opposites," verbal and physical aggression. In the 3-5 year olds studied, prosocial and aggressive actions toward peers were found to be complexly interrelated. They are modestly positively correlated in frequency so long as aggression does not exceed a moderate level. At high frequencies or intensities of aggression, however, the two kinds of interactions are inversely related. Among children who are themselves not very aggressive, frequency of being the target of aggression and frequency of expressing sharing and comforting are positively related. Among highly aggressive, highly victimized children, no predictions can be made concerning compassionate behaviors, which may be frequent or virtually nonexistent. This study is preliminary to more extensive investigation, now in a planning stage, of children's behaviors that deal positively with the welfare and feelings of other persons.

Significant distortions or limitations in the individual's awareness of other people's feelings are among the defining characteristics of psychopathology such as paranoia, sociopathy, or autism. Even "normal" individuals frequently have areas of insensitivity to the need states of others. The development within oneself of accurate empathy is not only an explicit task for parents, psychotherapists, and educators; it is a condition necessary for most people's successful day-to-day social functioning. It is therefore important to understand the developmental stages through which sensitivity and responsivity evolve and the various ways in which sensitivity and empathy are actively encouraged or discouraged in the course of different socialization experiences. Our evidence, for example, indicates that the capacity for compassion, for reaching out to others in a giving way, is present and active by the end of the first year and a half of life. The means by which this capacity is "lost," selectively dulled, or distorted in many individuals require careful study both as an important theoretical problem and as a question of major, practical social significance.

Summary

A word about the staff of the Laboratory: Members of the senior research staff are centrally involved in the scientific life of the NIMH-NIH complex--on internal review committees in several of the Institutes, on the Children and Youth Committee of NIMH, on the EEO Council, as president of the Assembly of Scientists, NIMH-NINCDS-NEI.

The research of the Laboratory has the benefit of an exceedingly skilled and research-oriented support staff.

The senior investigators carry responsible leadership roles in the discipline of psychology, expressed (a) in numerous invited addresses and conference participation in universities in the U. S. and abroad; (b) as elected officers in the APA, Division of Developmental Psychology, and APA Committee on International Relations; and (c) in memberships on advisory and editorial boards of principal scientific journals--Developmental Psychology, Child Development, Annual Review of Psychology, Human Development--and of specialized journals--Journal of Autism and Childhood Schizophrenia, Youth and Society, and Journal of Applied Behavioral Analysis.

In final summary, the research projects that have been reviewed in brief were all begun before the Laboratory came into administrative existence. We are this year reaping entirely the investments of the past. In many respects, we will continue to do so, but we hope in addition to see reflected in next year's review the initiation of new directions and an approximation of the Laboratory identity described in the introduction of this review.

Annual Report of the Laboratory of Psychology and Psychopathology

National Institute of Mental Health

David Rosenthal, Ph.D., Chief

July 1, 1974 - June 30, 1975

This Laboratory has gone through dramatic changes during the past year. Organized originally by Dr. David Shakow about 21 years ago, it has until April 1975 functioned under the name: Laboratory of Psychology. However, with the recent reorganization of parts of the Intramural Research Program, the realities of new developments and perspectives required that we revise our name to the Laboratory of Psychology and Psychopathology. This new name indicates more clearly the major focus of our current research goals and the substantive and methodological contributions of psychology and related behavioral sciences, e.g., behavior genetics, and personality theory and development, to the potential solutions or ameliorations of problems in mental health and illness.

During the past few years, we have gradually reduced the size of the Laboratory appreciably, not only because of budget cuts of which we are all aware, but also to keep the thrust of our research in sharper focus, and to marshal a small tightly knit group of researchers who can interact beneficially with one another and stimulate high motivation, collaborative participation, and new constructive ideas. In the process, we have abolished all Sections but one (Neuropsychology), since the other Sections had become much too small, and they tended to compartmentalize the Laboratory rather than to integrate it. The Section on Early Learning and Development was transferred to another reorganized Laboratory. Another concomitant of these changes is that independent investigators in the Laboratory (apart from Neuropsychology) are almost equally divided between M.D.'s and Ph.D.'s, and this balance works effectively to generate the kind of stimulation that should produce the most desirable research programs for this Laboratory. Of course, members of the Laboratory continue to collaborate with scientists in other laboratories and research centers, and they continue to carry on their own respective research projects of special interest to each. We are still very much in a transition period and the presentation of our research findings for the current year differ in some respects from those of previous years. These findings are as follows:

Cortical Mechanisms in Vision

In a continuing collaborative study with Gross at Princeton, we have delineated the functional pathway through which inferior temporal neurons receive their visual information. The method entails measuring the defects in the visual receptive fields of single IT neurons after selective cerebral lesions or commissural transections. As predicted from our earlier behavior-ablation studies, the subcortical pathway from superior colliculus through pulvinar to IT cortex has turned out to be unimportant for the receptive field properties of IT neurons. By contrast, the cortical pathway from striate through prestriate to IT cortex has proved to be essential, since interruption of this pathway by a unilateral striate removal or by section of the forebrain commissures eliminates the corresponding visual input to IT cells. Furthermore, of the IT neurons in each hemisphere that receive a visual input across the commissures from the opposite hemisphere, approximately half receive theirs exclusively through the splenium of the corpus callosum, whereas the other half can obtain their input either through the splenium or, alternatively, across the anterior commissure. Whether there is a difference in the kind of visual information that is carried in the splenium and anterior commissure is still unknown.

Since nearly two-thrids of IT neurons receive information from both hemispheres (i.e., from both the left and right visual half-fields), we have proposed that IT cortex plays an important role in stimulus equivalence. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we have prepared monkeys with bilateral IT ablations combined with section of the optic chiasm, and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either IT lesions alone or chiasm section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkeys must learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence between (and probably also within) the visual half-fields depends on the convergence of their projections onto single IT neurons.

This spatial convergence upon single cells provides a mechanism that is essential for discrimination learning. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in distance, direction and angle of regard that lead to stimulation of different populations of geniculostriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the IT cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Monkeys that are shown an object once will recognize it as familiar a few seconds later, as indicated by the fact that they will avoid it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus sets up a circuit, or trace, against which a subsequent presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be the anterior IT cortex, since removals here but not elsewhere abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, the anterior IT cortex contains the traces laid down by previous viewing, and these serve as models or images against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. A major goal of our future research will be to devise methods to study these newly localized visual traces at the single-unit level.

The evidence that has been cited favors a sequential activation model for object vision. Information that reaches the striate cortex is transmitted for further processing to the prestriate cortex, and from there to the IT area. Can this model be extended beyond vision per se to encompass stimulus-reward association learning? Translated into functional anatomical terms the question is whether visual objects might gain motivational and emotional significance through an interaction between the IT cortex and the limbic system. Some preliminary findings support this possibility. Earlier we had shown that equally severe deficits in object discrimination reversal learning are produced by bilateral removal of IT cortex and by bilateral removal of the temporal pole and amygdala (TPA), a limbic region to which the IT cortex projects. To test whether the similarity in outcome reflects a truly functional relation between the two areas, we prepared monkeys with an IT removal in one hemisphere, a TPA removal in the other, and a transection of the anterior commissure. The effect on object reversal learning was the same as that of the bilaterally symmetrical lesions, suggesting that section of the anterior commissure had disconnected the intact TPA region on one side from the intact IT cortex on the other. Section of the corpus callosum instead of the anterior commissure was ineffective; and section of the anterior commissure also was ineffective if the striate area was removed instead of IT cortex, presumably because in this case the intact striate and IT areas in one hemisphere were still connected with the intact IT and TPA areas in the other through the splenium of the corpus callosum. The results thus support the proposal that the process of associating visual stimuli with reinforcements depends in part on a visual-limbic pathway with a link in the IT cortex.

To determine directly whether the temporal pole and amygdala are involved in the establishment of stimulus-reward associations, we have initiated a study to measure the activity of single units in these areas while an animal performs on a visual discrimination reversal task. Positive evidence for our hypothesis would be provided by a finding that the discharge frequency of a given unit depends on the reward value of the stimulus (A+ vs A-) and not on its sensory quality (A vs B). The converse results, i.e., sensitivity to the sensory and not to the affective quality of the stimulus, would be expected of units in the IT cortex, for according to our model this tissue is an intrinsic part of the visual system and concerned with purely visual processing. Preliminary results have been gathered thus far on units in the IT cortex, and, as predicted, many of the neurons here discharge selectively to visual patterns--one cell preferring one pattern, another cell preferring another pattern, and so on--whereas none of these neurons appear to code the reward value of the patterns. Whether such a code is carried by neurons in the temporal pole and amygdala still remains to be determined.

Prefrontal Cortex

The neocortex, particularly those regions of it concerned with "associative" processes, achieves its highest evolutionary expression in man and sets him apart from all other species in terms of behavioral capacity. Unlike the motor and sensory regions of the cerebral mantle, the association cortex is panmodal and regulates functions that are many steps removed from the purely receptive or motoric aspects of behavior. Unraveling the complex and often elusive functions of this evolutionarily significant cortex is essential if we are ever to understand the quality and richness of man's mental and emotional life and the derangement of thought and feeling that occur in mental and emotional breakdown or disease. This Laboratory has therefore been devoted to the study of the structural and functional organization of the prefrontal association cortex in the adult rhesus monkey. The rhesus monkey has been an ideal subject for these investigations because of its phylogenetic proximity to man, because its brain is organized similarly to that of man, and because the size of its brain permits precise localization and intervention in functionally discrete regions. Previous research in this Laboratory has provided the necessary behavioral analysis upon which our current studies employing a variety of techniques are based. In this section we describe studies aimed at pharmacological and biochemical characterization of prefrontal cortical neurons.

Innervation of the cerebral cortex by aminergic neurons originating in the lower portions of the brain is well established, yet the physiological function of this innervation is not understood. Capitalizing on the known functional organization of the monkey's prefrontal cortex, we together with Drs. Charles Popper and Tim Havens, LCS, are exploring the behavioral effects of localized application of pharmacological agents to specific areas of this cortex. Chemical stimulation of discrete regions of the prefrontal cortex in the awake primate is possible by injection of small quantities of substances in isotonic saline solutions through surgically-placed in-dwelling cannulae. Behavioral observations can then be made on general activity and free behavior as well as on performance of cognitive tasks requiring the integrity of the prefrontal cortex. Preliminary findings indicate that norepinephrine, when injected in microgram quantities, is capable of eliciting hyperactivity and stereotypic movements similar to the behavioral changes often seen after systemic administration of amphetamine. The effect of norepinephrine on gross behavior is specific and is not elicited by dopamine or other punative neurotransmitters; further, the effect is produced by injections only into the cortex of the principal sulcus and not into the orbital prefrontal area, two regions of the prefrontal cortex that earlier work has shown participate in different behavioral functions. These studies may lead to the identification of a possible physiological role of norepinephrine in the frontal lobe, and a possible site of action of amphetamine in the cerebral cortex.

With Dr. Roger Mac Brown we are also involved in regional analysis of biogenic amine distribution and synthesis in various regions of the adult primate brain by measuring directly the levels of the monoamines, their metabolites and precursors in intact monkeys and in monkeys following acute and chronic interruption of specific frontal cortical pathways. Discrete areas of the brain that are of particular interest include the orbital and dorsolateral prefrontal cortex, hippocampus, amygdala, septum, caudate nucleus, hypothalamus, thalamus and brain stem. We anticipate that these studies will not only yield a description of the regional distribution of the biogenic amines in the primate brain but perhaps permit the identification of neurotransmitters in specific pathways from prefrontal cortical areas to subcortical structures known to be involved in selective behavioral functions. These studies will also serve as a basis for developmental investigations to be described in a later section of the report.

Development of Brain and Behavior

A major goal of this Laboratory continues to be the elucidation of the neural mechanisms responsible for the development of normal behavior and for the expression of plasticity in neuropathological development. Our approach is broadly multidisciplinary, involving behavioral, neuroanatomical, electrophysiological, endocrinological and biochemical techniques. Again, our experimental subject is the rhesus monkey, whose appropriateness as a model of human development is unexcelled. For studies of development, in particular, the protracted course of postnatal maturation exhibited by rhesus monkeys permits greater resolution of the timing, sequence, and correlation of structural and functional events than is possible, for example, in rodents or other laboratory animals.

Our previous work in this area has shown that specific regions of the primate cortex are functionally immature at birth and develop their functions according to widely different time-tables. However, the immature monkey differs from the adult not only with respect to cortical immaturity but with respect also to cortical-subcortical organization. This year, with the help of Dr. Garrett Alexander, we have utilized cryogenic cooling techniques and electrophysiological recording to further describe the changes in cortical-subcortical organization that take place in ontogeny. Previous studies by Dr. Alexander have shown that the frontal cortex can be inactivated in adult monkeys by cooling through cryoplates implanted over the dura mater of the prefrontal cortex. This technique has several advantages over the ablation technique including the avoidance of secondary degeneration in structures connected with the frontal cortex and also the possibility of functional effects that are reversible--allowing each animal to serve as his own control. In the adult monkey, localized cooling of the prefrontal cortex results in behavioral deficits classically associated with removal of this cortex but these deficits disappear as soon as the cortex is returned to its normal temperature. Furthermore, cooling has been shown to inhibit the firing of neurons in the dorsomedial nucleus of the thalamus in association with behavioral performance. Studies identical to those carried out in the mature monkey are now being conducted in normal monkeys at various postnatal stages of development. It is expected that the younger the monkey, the less functional inactivation of the frontal cortex will affect both behavioral performance and subcortical neuronal firing patterns. Analysis of the electrophysiological activity of subcortical (striatal and thalamic) neurons in response to cooling should reveal the extent to which subcortical mechanisms can function autonomously in immature monkeys, a notion derived from previous studies in this Laboratory, and one which may help to explain behavioral recovery after early brain injury.

Understanding normal changes in brain organization with age may help to explain certain manifestations of disordered development. Recently, the careful observations of Mr. Howard Crawford in our Laboratory uncovered a 6-month old monkey that displayed many characteristics of the hyperkinetic syndrome in man. The monkey exhibited incessant locomotor activity and pacing far in excess of that displayed by normal monkeys of this age and was unable to be trained on a conventional cognitive task routinely given to monkeys of this age. It has now been shown by Mr. Ken Jurist working with Dr. Brown that this monkey exhibits a clear paradoxical response to relatively high doses of amphetamine (0.8 to 1.6 mg/kg). With such doses, the monkey remains alert but sits uncharacteristically still during 30-60 minute observation sessions, though his activity during control sessions with placebo remains undiminished. This finding increases the probability that this infant may provide an animal model of the hyperkinetic syndrome. Further behavioral, pharmacological and biochemical studies are planned.

A large proportion of our effort this year had been devoted to examining the possible role that gonadal hormones may play in regulating the differentiation of cortical mechanisms. Previous work in this Laboratory has provided evidence that the orbital prefrontal cortex develops at different times in males and females. Currently we are studying the behavioral and biochemical consequences of postnatal injections of testosterone in developing female monkeys. Our postnatal injection regimen has so far produced both permanent morphological alterations in the external genitalia of these females and enhanced their ability over that of normal females to learn a task dependent on the orbital prefrontal cortex. Subsequent to further behavioral studies involving analysis of their psychosocial and sexual development, these monkeys will be sacrificed and their brains analyzed, along with brains of normal males, for distribution and biosynthesis of biogenic amines.

The possibility that there are neural differences between males and females at the cortical level under the control of gonadal hormones at critical periods in development is of extreme importance for understanding the normal differences in cognitive and social development of the two sexes as well as for understanding disturbances in psychosexual orientation that may occur as a consequence of exposure to an inappropriate hormonal milieu during gestation such as adrenogenital syndrome and progestin-induced pseudo-hermaphroditism. Future plans for work in this area include behavioral and biochemical studies of prenatally-androgenized females and prenatally feminized males (chemically gonadectomized with an androgen antagonist, cyproterone acetate). Prenatal studies are warranted because the peak sensitivity of cortical tissue to gonadal hormones may occur in the second trimester of pregnancy during which, in the primate, corticogenesis is completed.

In a somewhat related study, we continue to study Adam, a 2-1/2-year old monkey whose dorsolateral prefrontal cortex had been removed during the second trimester of gestation. Subsequent to intra-uterine surgery, the fetus was replaced in utero and delivered successfully at term (168 days' by caesarian section. Over the 2-1/2 years that the monkey has been observed, he has exhibited none of the behavioral deficits exhibited by monkeys sustaining injury to the same area in adulthood and his behavior was indistinguishable from that of normal monkeys at all stages of his development. Postmortem anatomical studies of his brain will provide definitive information on whether corticogenesis was complete at the time of surgery and whether thalamocortical connections had been formed at that time.

Anatomical Correlates of Development and Plasticity

Currently with the behavioral, electrophysiological and biochemical-endocrinological studies described above, we are conducting experiments aimed at elucidating the structural basis of normal and abnormal behavioral development. Our anatomical studies using silver impregnation techniques have extended and confirmed the findings obtained with behavioral and electrophysiological methods in indicating immaturity of the pathways from the orbital and dorsolateral prefrontal cortex to subcortical structures like the caudate nucleus up to 6 months of age and beyond. We are presently employing autoradiographic techniques to further amplify these findings in studies being conducted at M.I.T. under the supervision of W.J.H. Nauta. These studies should provide important information on the postnatal development of connections, a subject about which little is known. For example, to date there have been only two studies in primates concerned with the postnatal development of circuitry and neither of these has addressed the status of cortical-striatal or cortical-thalamic pathways.

A second major question that is being approached anatomically is that of the presumptive reorganization of the central nervous system subsequent to early cortical injury. Though central nervous system plasticity is presently an area of active research interest there has so far been no strong evidence to indicate the postlesion axonal growth or collateral sprouting is functionally adaptive. Our previous studies of behavioral recovery in monkeys sustaining orbital prefrontal injuries in infancy show that the monkey is an excellent animal model of behavioral plasticity in development and one highly suitable for studies of neural reorganization. We are therefore employing both the Fink-Heimer silver impregnation technique (in collaboration with D. Tanaka of Howard University) and autoradiographic methods to examine the brains of monkeys, now adults, that had received bilateral orbital prefrontal lesions as infants, for evidence of anomalous connections. These longitudinal studies, which are still in progress, should provide important information on whether behavioral recovery following early brain injury can be attributed to the capacity of the developing nervous system for postlesion axonal growth.

Cortical Mechanisms in Somesthesis

The general aim of the research is to clarify, by means of cortical ablations and behavioral tests, the functions and interrelationships of areas within and adjacent to the sensorimotor cortex of the monkey. As an adjunct, Drs. Turner and Semmes are using injections into selected cortical areas of horseradish peroxidase (a substance which is transported backward along the axon to the cell body) in order to identify thalamo-cortical pathways. In particular, we wish to determine whether or not area 2 (the posterior part of the postcentral gyrus, which electrophysiological studies indicate to be the main recipient of afferents from the joints and hence important to position sense) receives a projection separate from that of area 3. For this study, area 3 is removed from one hemisphere and area 2 from the other; 2 months later, when retrograde degeneration is sure to be complete, the remaining area 2 on one side, and area 3 on the other, are injected. Comparison of the thalami on the two sides should then answer our experimental question. Preliminary results indicate that area 2 does receive an independent projection that other histological methods had failed to detect.

There are two other experiments in which Drs. Turner and Semmes are collaborating. The aim of the first is to find out if the severe somatosensory deficit produced by unilateral lesions of the hand representation of area 3 c be duplicated by removal of the principal areas to which area 3 sends efferents (i.e., area 4 and 5 on the lateral surface, the supplementary motor area on the medial surface, and the second somatosensory area in the parietal operculum). It appears that such ablations do not interfere with any of a variety of somatosensory discriminations thus indicating that cortico-cortical interactions are not crucially involved in the functions disturbed. The aim of the second is to find out if the area 3 deficit is primarily one of detecting stimulus differences or of responding appropriately to such differences. A bimanual task is employed in which one hand palpates the stimuli and the other hand responds differentially. We found that it is only the palpating hand that is affected by a unilateral lesion in the postcentral gyrus of the opposite hemisphere.

Work is continuing on the contralateral effects of ablating various parts of the sensorimotor cortex, and the new behavioral findings will be considered together with those we reported previously. To date, the effects of each of fifteen different small lesions of this cortical region have been studied with our battery of tests; the battery includes discriminations of hardness, shape (at several levels of difficulty), size, and roughness, with the last two followed by determinations of differential thresholds. In contrast to the paradoxical results that have been reported in the literature (i.e., only slight and transient defects following bilateral removal of the main somatosensory projection area, the postcentral gyrus) our findings are in conformity with anatomical and electrophysiological data on the density of projection from the thalamus and the functional properties of the populations of neurons making up the distinguishable areas. The conception of parietal lobe function that emerges from our studies is that area 3, located in the posterior bank of the central sulcus, is by far the most important part, since lesions confined to this area have devastating effects on learning and performance of virtually all the discrimination tasks. The remaining areas of the postcentral gyrus, areas 1 and 2, represent cutaneous and proprioceptive sensibilities separately, with the former chiefly dependent on area 1 and the latter chiefly on area 2. Lesions anterior or posterior to the postcentral gyrus (that is, outside the sensory projection areas) do not produce deficits on our series of tests. As reported last year, removal of an area ventral to the postcentral gyrus--the parietal operculum, which has been identified electrophysiologically as the second somatosensory projection area--does produce a deficit, although it is less severe than that following lesions of area 3 in the postcentral gyrus, identified (together with areas 1 and 2) as the first and better-known projection area. Thus, it appears that only those areas receiving somatosensory afferent fibers via the recognized ascending pathways are crucial to somatosensory discriminations. It is noteworthy that discrimination tasks we employed, which varied widely in submodality type and in level of difficulty do not depend on the integrity of areas (such as the posterior pariental region) that have been thought to process and integrate the "raw" somatosensory information following its arrival at the cortical level.

These generalizations should be qualified as follows. A lesion of area 3 does not produce a deficit in all somatosensory functions, since it does not appreciably impair punctate tactual sensitivity, or fine temperature discrimination, or a very easy size discrimination. Since we have previously shown that, even with area 3 intact, deficits can be produced by removal of area 1, area 2, and the parietal operculum, as well as by massive lesions of the ipsilateral sensorimotor region or of the contralateral frontal or temporal lobes (with respect to the hand tested), area 3 is obviously not the only part of the cortex involved in somatosensory discriminations, although it is clearly the most important. Another qualification relates to the tests we employed. Fine roughness discrimination is vulnerable to almost every large cortical lesion, and to small lesions of the posterior parietal region of the hemisphere contralateral to the tested hand. This test appears to be too sensitive, or alternatively, it may be dependent on too many factors (perhaps some of which are not sensory) to allow assessment of the relative importance of various cortical areas. Still another caveat should be expressed: in a strict sense, our conclusion that only the somatosensory projection areas are crucial to normal learning and performance of somatosensory discriminations is specific to the size and locus of the lesions we made and to the particular tests we employed. In fact, another way of looking at our results is that our battery of tests taps mainly the functions served by the projection areas. Thus, other tests might well reveal deficits on somatosensory tasks after lesions of the areas adjacent to the projection areas, at least. Indeed, such results have already been reported, and we do not question their validity; however, it is clear from the present findings that the sensory projection areas, as defined anatomically and electrophysiologically, can also be identified as uniquely important by the method of selective ablations and behavioral tests.

Schizophrenia and Psychopathology

During the past four decades there has held sway a persistent belief that schizophrenics and persons with a schizoid character engage in migration more often than do non-schizophrenics. A study in this Laboratory provided strong evidence that this prevalent belief is probably not true as such, that people genetically related to a schizophrenic may actually be less likely to emigrate than are individuals not related to a schizophrenic, and that some personality factors such as anhedonia, passivity, dyssociality, and declining intellectual organization may be contributing to a lower than normal migration rate in such persons.

A second study assessed the association between the quality of parent-child relationship and the degree of psychopathology in the child. Four groups of subjects were involved in the study. Three of the groups were adult adoptees who either had a schizophrenic biological parent, or a schizophrenic adoptive parent, or biological parents with no known psychiatric disorder. A fourth group consisted of individuals who had a schizophrenic biological parent and were reared in the parental home during at least the first 15 years of life. All Ss had a detailed psychiatric interview which was summarized and placed on a card. Judges independently evaluated the quality of the relationship between the S and his rearing parents by reading all the cards and deciding on which Ss had the best to the worst relationships respectively with their rearing parents. Judges, using a similar procedure, also read diagnostic statements made by the interviewing psychiatrist in regard to each S and independently ranked the Ss in terms of which had the least to the most psychopathology respectively. The rankings of parent-child relationship and degree of psychopathology in the child were then correlated. Reliability of both measures was high. It was found that there was indeed a statistically significant correlation between the two measures. However the correlations were low, running from 0.210 to 0.285. The amount of the variance accounted for in the correlations ranged between 6.5 percent and 9 percent. The groups could be divided into a "genetic group," in which each S had a schizophrenic biological parent, and a "non-genetic group," which did not have a schizophrenic biological parent, but part of them may have had an adoptive parent who was schizophrenic. The correlation between the two measures in the "genetic group" was 0.266, but 0.366 for the "non-genetic group." The amount of explained variance was only 5.1 percent for the "genetic group," but 13.4 percent for the "non-genetic group," almost a threefold difference. The findings suggest that rearing patterns have only a modest effect on individuals who harbor a genetic background for schizophrenia, but an appreciable effect on persons without such a familial background.

Another study assessed the psychophysiological functioning of adopted-away offspring of schizophrenics (index cases) as compared to adopted-away controls. The index group produced significantly larger amplitudes in response to an orienting tone, and a greater frequency of responses to the unconditioned stimulus (UCS) as compared to the controls. The index group showed lower habituation of responses to the UCS than the controls. No other comparisons were statistically significant. The findings suggest only a weak relationship between an inherited diathesis for schizophrenia and electrodermal reactivity.

Data collection has been completed in a study on drug-free acute schizophrenic patients using procedures which assess the tonic and phasic activity of several peripheral autonomic variables under conditions of rest, mild auditory stimulation and on a test of attention. The tests were done soon after admission, just before discharge and 6-12 months after discharge. Data analysis shows that on admission the patients, compared to normal controls, were significantly higher on several measures of autonomic activation but lower in phasic responsivity to both sensory stimulation and task-related stimulation as well as in the phasic response to task performance. Despite non-significant differences in admission ratings of psychopathology, the patients who were going to improve by the time they were discharged were lower on several indices of autonomic activation and higher in responsivity than those patients who were no longer acutely psychotic, had normal activation levels, but remained abnormally low on some responsivity measures. Further analysis will focus on 6-12 month follow-up data and correlations of the autonomic data with detailed behavior ratings of the subjects.

From the data analyses done to date it seems as if most of the differences found between acute schizophrenics and controls are due to the presence of an acute schizophrenic state rather than representing permanent "traits," although many seem to have at least short-term prognostic significance. However, some of the variables reflecting low specific autonomic responsivity are possible indicators of a schizophrenic predisposition. Data from a large sample of monozygotic and dizygotic twins will be analyzed to see if these variables have a genetic basis. In studies in which subjects were tested daily for several weeks we have shown reliable changes in autonomic reactivity accompanying day-to-day changes in self-rated mood.

Another study produced the following findings:

(1) The IQ of the offspring of schizophrenics, especially the offspring of parents with chronic forms of schizophrenia, is lower than that of controls at age 4 years. It is also lower, but not significantly so, at age 7 years. The findings below apply to this chronic group.

(2) Among the offspring of schizophrenics there is a lower correlation between socio-economic factors and IQ than there is for the control population. In other words, socio-economic factors are poorer predictors of IQ in this group.

(3) Among the offspring of schizophrenics certain perinatal factors are more highly correlated with IQ than among the controls. Edema, vaginal bleeding, spinal anesthesia at delivery and mid-forceps delivery are quite highly correlated with IQ in a negative direction. In other words, these factors are predictive of a lower IQ within the offspring of a schizophrenic group.

(4) The higher correlations mentioned above could represent an effect of pregnancy and delivery complications. We found that these negative correlations were present whether it was the mother or the father who was the schizophrenic parent. This finding would indicate that there is a genetic susceptibility in the infant, not a unique effect of the schizophrenic mother.

(5) Among the offspring of schizophrenic mothers, there is a low correlation between 4 year and 7 year IQ, indicating that there has been some significant effect, either biological or environmental, between these two ages.

A study has been carried out on the implications of the genetic spectrum concept of schizophrenia for psychiatric diagnosis. Problematic areas have been identified with respect to the broadening of the schizophrenia ambit. However, research and practice have been distinguished, and it has been concluded that these problems inhere not in the concept itself, nor in the research methodology, but in the attempt at applying the concept in ways which are not yet supported by research or clinical findings.

Dr. Walter Reich, who regretfully, from our standpoint, will be leaving this Laboratory at the end of this fiscal year, has served for two years to consider the humanistic aspects of medical and mental health practices, and ways in which to improve them and tailor them to modern concepts. Among his observations and recommendations, the following are briefly noted:

The problem of the patient-physician contract has been examined from a psychological perspective. It has been concluded that the concept of physicianly responsibility derives to a considerable extent from professional forces--acting on the physician during his years of training as well as during his years of professional practice--which result in the development of a concept of "duty" designed more to satisfy the needs engendered by those forces than the needs of the patient. Some conclusions are drawn with regard to changes in medical education and patient-physician interactions.

Development of a Center for Social and Policy Studies at NIMH

A proposal for such a center was developed and discussed at meetings with NIMH researchers and administrative personnel. It was conceived as satisfying a number of recommendations made by the NIMH Research Task Force, including: (1) the call for more studies of social factors bearing directly on mental health and on the causal conditions of social problems; and (2) the establishment of a Culture and Social Structure Section and a Sabbatical Center. The proposal continues to be reviewed, with the likelihood that courses on these areas, and possibly a Sabbatical Center, will be instituted.

Proposal for a Course in Mental Health Research Administration

This was prepared for the Research Advisory Group, at the request of the Staff College. It presents the rationale for such a course, and suggests an outline for teaching it. It is assumed that the audience will include NIMH intramural and extramural staff, as well as staffs of departments of psychiatry, psychology, and social work; of community mental health centers; of private hospitals; of private research institutes; and of other organizations with an interest in mental health research.

We are continuing our research on speech behavior, with particular reference to the nature of the units that the speech processing system operates with, both in production and perception. A considerable body of accumulating evidence strongly suggests that the phonemic clause, a linguistically defined unit of 7 or 8 syllables on the average, is a functional unit in both production and perception.

On the perceptual side, the existing evidence was derived by a former member of this Laboratory, who showed that "attention responses"--head nods and mhms--are produced by listeners at the ends of complete phonemic clauses, which suggests that it is these temporal patterns or chunks that are being comprehended, and not individual words.

In a new approach to this problem we are currently studying listeners' attention by means of a different strategy, the cortical average evoked response This strategy depends on the fact that the cortical electrical response to an irrelevant stimulus, light flash, for example, is attenuated when the subject's attention is directed inward to cognitive processing.

By means of this strategy we hope to show that the perceptual processing of speech involves bursts of cognitive activity at successive phonemic clause boundaries. The experimental determination involves the comparision of the mean response to punctate stimuli timed to occur at clause boundaries with the mean response to control stimuli timed to occur elsewhere in the clause.

The results to date are equivocal but encouraging. An initial group of subjects showed the predicted difference, but the data analysis also revealed some methodological difficulties which are now being remedied.

It is important to note that, if successful, this project will open an unexplored domain. No investigation of brain response to continuous speech has ever been carried out. Beyond the initial normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular regard to lateral specification of cortical function. It has been suggested that much of the cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically an impairment in the capacity to filter complex stimulus patterns and focus on the salient elements. Since the left hemisphere seems to be specialized for speech, a psycholinguistic approach to the problem of laterality and attention has considerable strategic potential, at this stage of our knowledge.

Effects on time judgment have been found in schizophrenia, various drug conditions, and conditions of altered metabolic activity. The perceptual process involved cannot be assessed directly but rather must be inferred from a theoretical model which assigns sources for the variations in behavior that occur under specified conditions. The validity of the measurement thus depends on the adequacy of the model. We have concentrated on developing a model of the "internal clock" in terms of which effects on the time sense can be distinguished from cognitive and motivational effects which are likely to affect test performance without necessarily affecting the capacity to perceive time.

The model has previously been tested on judgments of short time intervals under conditions of depressed arousal. Subjective time rate decreased moderately, but there was also an increased tendency toward indiscriminate responding on the part of the subjects which by conventional measures of time estimation would be taken for a marked decrease in rate.

This year the model was tested on judgments of longer time intervals and with a nondepressant drug, THC. As with short time intervals, basic theoretical requirements of the model were met. Contrary to previous reports of distorting effects of THC on the time sense, performance was somewhat improved under the drug condition. The improvement, however, can be attributed to increased motivation on the part of the subjects to perform the task well rather than to an enhanced ability to discriminate time intervals accurately.

Objectively accurate time perception is a basic requirement for competent behavior and may be impaired in severe mental disorders. Results with the present model suggest, however, that the perceptual capacity itself is not as easily affected as commonly supposed.

Although ordinarily highly stable, the perceptual system is capable of adaptation to changes in stimulation that persist over time. To what extent such perceptual adaptation is a purely sensory as opposed to a conditioning process remains a major issue in perceptual theory. In order to investigate this question more critically, an apparatus for presenting combinations of visual stimulus motion, color, and form has been constructed and is being tested. Adaptation occurs to these variables singly, but conditioning may be necessary to produce adaptation to combinations of stimuli. If so, a fundamental form of learning must be considered necessary in perceptual development and adjustment to environmental stimuli.

Cognitive Functioning

The mental processes that are responsible for resolving the proble atic situations that people encounter in their daily transactions with their environment have been investigated for several years by this Laboratory. This research is based on experiments in which people conduct inquiries by interacting with well-behaved systems controlled by computer programs. The results are used for three purposes: (1) to assess the methods commonly used by peopl in searching for solutions to puzzling situations; (2) to evaluate psychological theories that purport to explain this aspect of human behavior, and (3) t develop methods of improving people's ability to cope with the problems of daily life.

A study on attribute identification by the selection method was carried out during the year. In addition to describing an improved procedure, we demonstrated a method of assessing, in terms of information theoretic indices, the objective complexity of concept recognition tasks. Related adjustments to performance scores also permitted separate evaluation of two formerly confounded cognitive processes: that of accessing information and that of interpreting it. This analysis, in turn, suggested that two formerly opposed theories of cognitive complexity are in fact complementary. Specifically it indicated that concepts classified as cognitively simple by the hierarchical theory of conceptual difficulty generate relatively complex uncertainty structures, whereas concepts rated complex by this theory give rise to relatively simple uncertainty structures. As a result the two factors of cognitive complexity and uncertainty structure counterbalance one another when performance is evaluated in terms of raw scores. The separation of processes revealed that information was accessed quickly in the cognitively complex (low uncertainty) tasks, but was interpreted with difficulty, whereas the reverse was th case in cognitively simple (high uncertainty) problems.

A second study deals with the ability to recognize relevance. In ma real life situations and, unfortunately, in the traditional laboratory paradig used for studying concept identification, it is difficult to decide whether a person has simply guessed correctly, or has rationally identified the relevant aspects of the problem situation. This experiment was designed to enable us to detect those Ss who, instead of attempting to guess, made reasoned use of cues to relevance. The results indicated that although guessing was the dominant approach initially, many people soon learned to use the relevance cues with near maximum efficiency. Even more important than this basic finding was the indication that the ability acquired while working on one problem form was apparently transferred to other forms quite effectively, thus suggesting that this highly abstract cognitive ability can be improved with training.

All the basic procedures used in this area of investigation can no be completely controlled and executed by computer software. This kind of im mentation has several advantages: (1) procedures that would be prohibitively complex by other means become feasible. (2) S can work in complete privacy, free of the disturbing presence of the investigator. (3) He can be given complete control of pacing with no inconvenience to E. (4) Any aspect of the testing process can be easily recorded; e.g., only a fraction of a millisecond is required to record an item like response time. (5) Summary progress reports can be obtained at several design levels. (6) New experiments can be implemented easily--often by software modification alone.

Human Genetics and Environments

Dr. Gordon Allen, a geneticist, has transferred to this Laboratory from the Laboratory of Socio-Environmental Studies, and he brings with him a large study which he has been carrying out for several years.

Most of his work this year has been devoted to the statistical analysis of data obtained in the 1967 survey of Old Colony Mennonites, which was designed to demonstrate forces of natural selection with respect to human behavior. Social, economic and cultural items were nearly all reduced to three global categories: wealth, industriousness and social integration, for each of which two or three orthogonal factors could be defined. There is some overlap among the categories.

The results of this analysis have been added to the individual case "Census records," which he is now linking with detailed vital records of each family kept by the Church. For many households not reached by the census he has data that were collected in a government "land survey" which can also be linked to the church's family records. It will then be possible to detect and reconcile discrepancies between data from the several sources.

When these linking processes are complete, he plans to derive a set of statistics that will describe any significant relations between the behavioral variables and such vital measures as survival and fertility. Hopefully this may contribute some new information on population dynamics, in addition to delineating selective forces that acted upon behavior during 1000 years of European peasant culture.

Two other research projects of his are entirely in the planning stage: (1) A search for correlations, possibly genetic, between parental and infant behavior. This research will utilize the data of the Child Research Branch. That analysis is awaiting the collation of the files now going on in the CRB. (2) New attempts have been made in the past month to obtain data on fertility of the mothers of twins in the Collaborative Study of Cerebral Palsy. This project was initiated several years ago but has been dormant because the Perinatal Research Branch was not prepared earlier to yield the data.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES

National Institute of Mental Health

July 1, 1974 through June 30, 1975

Melvin L. Kohn, Ph. D., Chief

This Laboratory has been so radically changed that it is almost a new entity. Over the past two decades, the Laboratory of Socio-environmental Studies, while never large, has always been diverse: for most of its existence, its eight or ten principal investigators spanned the disciplines of human genetics, developmental psychology, social psychology, sociology, and anthropology. Its strength lay in that very diversity: people of different theoretical perspectives, having different methodological strengths and predelictions, informed, influenced, and critiqued each other's work, to everyone's advantage. With the death of one principal member of the Laboratory, the departure for university positions of two others, a shrinkage of resources that prevented their replacement, and an Intramural Research Program reorganization that has assigned human genetics and developmental psychology to other Laboratories, the Laboratory is now much smaller and more homogeneous. We are now only four senior investigators, all sociologists or social psychologists. But although we have lost size and diversity, we have retained our strength in what has always been the core of our program, the study of the processes by which social structure affects personality and behavior. And we are adding fresh talent in the persons of two post-doctoral fellows and a visiting scientist--something we have not been able to do in recent years. Thus, the prospects for the "new" and more sharply focussed Laboratory of Socio-environmental Studies seem favorable.

The following vignettes summarize the past year's work on the major projects currently underway in the Laboratory.

OCCUPATIONAL EXPERIENCE AND PSYCHOLOGICAL FUNCTIONING

The principal goal of Melvin Kohn and Carmi Schooler's research on the social psychology of occupations is to assess the effects of occupational conditions on psychological functioning. The research, up to now, has been based on structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted for us and to our specifications by the National Opinion Research Center of the University of Chicago in the spring and summer of 1964. During this past year, the same Center conducted follow-up interviews, again to our specifications, with a randomly selected one-fourth of the men who had participated in the original survey. If a man was found to be presently married, a nearly identical interview was conducted with his wife. And if a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a randomly-selected child.

The full rationale for this survey was presented in last year's annual report. Essentially, the follow-up study of the men is designed to provide more definitive data on causal processes than could be provided by a single cross-sectional survey. With these data, we hope to demonstrate that occupational experiences really do affect psychological functioning, that the correlations between the two are not just a statistical artifact. The study of the wives is designed to discover whether occupational conditions affect men and women similarly, and to enable us to assess the effects of men's occupational experiences on their wive's psychological functioning and of women's occupational experiences of their husbands' psychological functioning. The study of the children is designed primarily for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development. It also provides a basis for analysing the effects of educational and occupational experiences on their own psychological development.

The principal achievements of this past year--and certainly the principal preoccupations of the investigators--have been the successful completion of fieldwork, the development of an elaborate schema for coding the data, the actual coding of the data, and the development of an even more elaborate computer program for checking out the accuracy of the codes. Now, and for an as-yet unpredictable time to come, we shall be using this program to check the accuracy of the codes and to rectify all coding errors that we can find.

Simultaneously with the development of the current study, we have completed two further analyses of the original (1964) data, one (by Kohn) on the relationship between occupational structure and alienation, the second (by Schooler) on the effects of ethnicity on values and psychological functioning.

Occupational structure and alienation.

Before one can search for the occupational sources of alienation, he must deal with the fact that alienation is an extraordinarily vague and imprecise term. Lacking an adequate general definition, we follow Seeman's example and use the term to refer generally to five distinguishable facets of orientation: powerlessness, self-estrangement, normlessness, cultural estrangement, and meaninglessness. Seeman's analysis demonstrated that these meanings of alienation are so disparate that the term cannot be used analytically without specifying which meaning is intended. We thus attempted to develop indices of all five types of alienation. We actually succeeded in constructing adequate Guttman Scales of four of them, all except meaninglessness, which we could not empirically differentiate from the others.

Essentially, the strategy of analysis is to winnow down a preliminary inventory of more than fifty separable dimensions of occupation to those few that are significantly related to one or another aspect of alienation, even when education and all other pertinent facets of occupation are statistically controlled.

Only six of the 50-odd occupational conditions we studied have a significant, independent impact on powerlessness. These six do not include ownership, nor even what is often taken to be the modern equivalent of ownership, position in the supervisory hierarchy. Nor is division of labor conducive to feelings of powerlessness, for employees of highly bureaucratized firms and organizations are somewhat less likely to feel powerless than are employees of non-bureaucratic firms and organizations. Thus, the occupational conditions classically believed to be the source of powerlessness are not, under modern conditions, crucial to this form of alienation. Instead, three types of occupational experience stand out: low pay; too close supervision and too little substantive complexity to allow the worker to be self-directed in his work; and the threat that he might lose his job or business.

Two of the three principal occupational conditions that bear on powerlessness--a lack of opportunity to be self-directed in one's work and the threat of losing one's job or business--are important for self-estrangement too. But there are differences as well: Income is not significantly involved in feelings of self-estrangement; another threat, that of being held responsible for things outside of one's control, is pertinent for self-estrangement. Thus, the occupational conditions underlying powerlessness and self-estrangement are variations on a common theme. Centrally important to both are the conditions that make for occupational self-direction.

The occupational conditions pertinent to normlessness are, once again, mainly those that determine whether or not one can be self-directed in one's work: closeness of supervision, routinization, and substantive complexity.

Cultural estrangement presents an altogether different picture from powerlessness, self-estrangement, and normlessness, both in terms of which occupational conditions are involved and how they are involved. Ownership is significantly involved; bureaucratization is not; occupational self-direction is less important than for the other types of alienation. A new uncertainty, that there might be a "dramatic change" in one's income, reputation or position, enters in; so, too, does the time-pressure under which one works. In general, then, the overlap between occupational conditions related to powerlessness, self-estrangement, and normlessness and those related to cultural estrangement, is insubstantial. And where there is overlap, the direction of effect is opposite: working at jobs of low substantive complexity, for example, is related to feeling powerless, self-estranged, and normless, but to not feeling culturally estranged. One would have to conclude that this type of alienation is not produced by anything like the same occupational conditions as are the other three.

For powerlessness, self-estrangement, and normlessness, though, one can speak of similar underlying conditions, with a lack of opportunity for occupational self-direction being central to all. Thus, the occupational conditions underlying powerlessness, self-estrangement, and even normlessness center on the modern determinants of control over one's essential job conditions. These are no longer ownership and division of labor, but closeness of supervision, routinization, substantive complexity, and a basic sense of job security.

Ethnicity and psychological functioning.

The data of the occupations study were also used to examine the effects of ethnicity on values and psychological functioning. These analyses ask whether ethnic groups can be conceptualized as existing along a meaningful continuum that reflects social-structural conditions that are known to affect individual psychological functioning. The general hypothesis was that members of ethnic groups that originated in European countries having a relatively recent history of feudal subjugation are different in their intellectual functioning, attitudes toward authority, and moral value systems from members of ethnic groups coming from European countries where the institutions of feudal serfdom were terminated in the more distant past.

In line with this hypothesis, the major European nations groups were arranged along a continuum reflecting the time of the release of the peasantry from serfdom. Although this continuum is based only on the year that serfdom was ended, it actually reflects both the degree and recency of a larger complex of legal, economic, and occupational constraints on the lives of the mass of pre-industrial agricultural workers. Since this web of interrelated constraints on the individual's independence occurred in the diverse contexts of different national histories, the present ranking--based as it is on the different times of the ending of legal serfdom--does not perfectly reflect the exact chronology and severity of the various other components of this web of constraints. However, such a ranking does provide a reasonable and nonarbitrary way of ordering European countries according to the general magnitude and recency of constraints placed upon the autonomy of the individual peasant.

In general, the expectation that ethnicity--thus indexed--will have a generally linear effect on intellectual functioning and social orientation is confirmed, even with age, father's education, religion, rurality, and region of the country statistically controlled. Thus, individuals belonging to ethnic groups originating in countries with a longer history of freedom from serfdom show a higher level of intellectual flexibility and make more intellectually demanding use of their leisure time. The predictions about attitudes toward authority also prove to be correct: Members of ethnic groups stemming from countries at the end of the continuum relatively remote from serfdom are less authoritarian and more likely to have self-directed, rather than conforming, values. Evidence that individuals from ethnic groups long free from serfdom are more likely to value moral autonomy is found in such individuals' tendency to hold more personally responsible standards of morality, to believe themselves more responsible for their own fate, and to have a more self-deprecatory/self-critical view than do members of ethnic groups more recently emerged from feudalism.

Present-day ethnic differences thus appear to be congruent with the assumption of an historically determined continuum that reflects the social, legal, economic, and occupational conditions of the European countries from which American ethnic groups emigrated. Underlying the tentative tone and conditional tense of this conclusion are two lingering doubts: (1) Is ethnicity the actual and nonspurious cause of the differences we have found? and (2) If it is the true cause, are the interrelated legal, economic and occupational correlates of a recent history of serfdom really centrally involved in the causal processes leading to these psychological differences? The answer to the first question--have we found true ethnic differences--is very probably "yes." The various controls used rule out the possibility that our results are artifacts of differences in rurality, region, age, religion, father's education, present social class and the substantive complexity of the present occupation. If the ethnic differences are spurious, they would have to be the result of something outside of this wide range of variables.

The answer to the question of the centrality of the conditions related to a history of serfdom to the differences among American ethnic groups must remain somewhat tentative. However, here, too, certain alternate hypotheses may be ruled out. The ethnic continuum does not directly reflect climate, time of industrialization or time of urbanization of the old country, time of major immigration to the United States, or the social class backgrounds and motivations of the immigrants. It would therefore seem likely that we are, in fact, dealing with the remnants of cultural patterns originating in the agricultural sectors of the old country, carried over by the immigrants and carried on, to some extent, by their descendants. Thus, although no longer serfs, Americans from ethnic groups with a long and pervasive history of serfdom appear heirs to the cultural transmission of traditions whose values were molded by the constrained lives of their ancestors.

THE SOCIAL-STRUCTURAL SOURCES OF STRESS

Leonard Pearlin's inquiry into the social-structural sources of stress is based on interviews with a representative cluster sample of 2300 adults in the urbanized area of Chicago. The ultimate purpose of the study is to understand the daily, recurring problems and strains experienced by people of different social backgrounds, the emotional stresses and psychological disturbance that may result from these strains, and the coping strategies that are used to deal with these difficulties. A guiding perspective of the study is that many important experiences of people are organized around their structural locations in the society at large, and when these experiences are conflictful, threatening or frustrating, they become sources of inner distress.

Much of the work of the past year has centered around three problems: the stresses arising within marriage; sex differences in depression; and the use of alcohol as a coping mechanism.

Marital stress. This inquiry, begun in the previous year, is primarily concerned with the emotional consequences of marriage between partners of unequal status backgrounds. When status advancement is important to the parties of such marriages, a number of strains that eventuate in stress are likely to appear in their marital relations. People who have married down but to whom status aggrandizement is important are likely to experience disruptions in communications, in the sharing of values, and in the exchange of affection. Such disruptions in turn, are likely to result in a high level of emotional stress. This process is especially likely to occur when marrying down is coupled to striving for upward mobility.

Sex differences in depression. Women were found to be considerably more prone than men to depressive symptoms. This finding is consistent with those of many other studies. The present study was able to go beyond existing work by testing some of the possible reasons for the differences. One widely accepted hypothesis is that women are more vulnerable to depression because their role choices are more restricted than are those of men. In particular, women who are homemakers do not always have the freedom to choose outside employment. But this cannot account for the observed sex differences in depression, for women employed outside the home are no less inclined to depression than are homemakers.

It is not the fact of employment inside or outside the home, but the quality of experiences within the roles, that is important for depression. For example, homemakers exposed to the relentless demands of large and young families are more prone to depression than are homemakers with fewer and older children. Similarly, women employed outside the home who have difficulty in accommodating their jobs to marital and parental roles are more likely to suffer from depression than are women whose work is not in conflict with family obligations. The analysis indicates how a fundamental psychological state is influenced by the organization of experience within social roles.

Drinking as a coping mechanism. It is apparent that alcohol is consumed by some people as a technique for managing stress. Pearlin's analysis establishes the presence of a network of conditions leading to the use of alcohol for this purpose. The analysis particularly emphasizes economic factors, showing that limited material resources tend to generate a high level of anxiety that, in turn, leads to drinking as a device to keep the anxiety within manageable bounds. Drinking is especially likely to be used as an anxiety control by people low in self-esteem, who have a limited sense of mastery, and who do not have extended educations. The inquiry reveals the intricate connections between the distribution of economic resources, personality factors, psychological distress, and the practices people adopt to deal with their distress.

Two additional analyses are currently underway. One concerns a frequently observed fact: married people display a greater sense of psychological well-being than do either the never-married or the formerly-married. This is not a newly discovered fact; past work, either explicitly or by suggestion, has attempted to explain this difference by presuming that those without mates suffer a psychic deprivation. However, there are other circumstances differentially associated with marital status, such as social isolation and economic hardship, and preliminary results indicate that these circumstances, rather than the sheer presence or absence of a spouse, create difficulties in psychological functioning.

The second analysis is broader in scope than any undertaken thus far. It seeks to observe relationships among the entire range of variables included in the data. In so doing, it will attempt to recreate statistically the process that joins the social characteristics of people, the daily strains they experience in the different areas of their lives, the stresses they feel and the anxiety and depression they harbor, the coping resources available to them, and the repertoires of coping resources they employ.

Finally, preliminary plans have been made to reinterview the original sample. This extensive follow-up study would attempt to predict from what is presently known about their existing coping dispositions how people would respond to new strains in their lives. The follow-up is being planned in cooperation with investigators from the Committee on Human Development of the University of Chicago.

HEALTH ORIENTATIONS OF CHILDREN

The central issue in John Campbell's research is to learn how children acquire and modify their perspectives on illness and health. The study is based on focused interviews and questionnaires provided by a sample of 264 hospitalized children and their mothers, as well as ratings of the children's behavior by attending nurses.

Previously reported analyses have shown that children's age and sex, fathers' socioeconomic status, and mothers' valuation of self-direction are generally associated with variations in children's reports of their own health-relevant role functioning. Analyses completed this year have clarified and added to an understanding of the processes by which such self-appraisals develop.

Socialization research has conventionally used the father's socioeconomic status as a measure of the entire family's social position. But the possible independent contributions of the mother's own status should also be considered. This research finds that maternal and paternal status have generally similar, but not always equally strong, impact on children's views of their own behavior. Risk taking (i.e., the extent to which the child indicates that he takes chances that might result in injury and the extent to which he says he departs from a number of prescribed hygienic practices) represents an aspect of the child's actions least likely to be subjected to direct maternal scrutiny. Here the father's status plays a markedly more important role than does that of the mother; the higher his status, the greater the child's reported risk taking. When it is a matter of children's emotional response to circumstances surrounding illness itself, the influence of the mother's status is clearly greater than that of the father; the higher her educational level, the lower the level of emotionality reported by the child. Responsibility for tending the sick child typically devolves on the mother, and status-linked aspects of her functioning as caretaker may help determine the nature of her child's approach to the sick role.

Parental values provide a subtle, but pervasive, climate for the child's development. Two such values, each statistically independent of the other, have been measured in this study: (a) the importance the mother attaches to the child's self-direction, rather than conformity, and (b) maternal valuation of the child's egocentric, rather than sociocentric concerns. These values are themselves a function of attributes both of parents and child: Mothers are much more likely to value self-direction for older children and for boys; and they are also more likely to value self-direction if their own and their husband's status are both high. Sex of child and maternal status are primary contributors to the second of these values: Mothers who are less educated and whose child is a boy attach much more importance to egocentric than to sociocentric concerns; age of child and father's status have no appreciable impact on this value.

Children's appraisals of their own role performance appear to be much more closely related to their own and to their parents' statuses than to their mothers' values. The lesser contribution of the values might simply suggest that its members' various statuses summarize far more about a family climate than is conveyed by the two values measured. But the situation is more complicated than that. Variations in the importance that mothers attach to self-direction and to egocentric concerns _do_ make a difference. They contribute quite significantly to children's role assessments, but they do so in a conditional manner: their effects are markedly moderated by the age of the child--the older the child, the greater the impact of maternal values.

Separate multiple-regression analyses show that value-by-age interactions make significant incremental contributions to each dimension of children's role assessments; both of the values contribute to these increments. Among older children, those whose mothers attach importance to the child's self-direction characterize themselves as displaying less emotion in illness, making their own decision concerning adoption of the sick role, and taking greater health-relevant risks. Older children whose mothers place a relatively low value on the child's egocentric concerns are also likely to see themselves as risk takers, and as the ones who make their own decisions concerning adoption of the sick role; in addition they characterize themselves as acting in a manner indicating rejection of the sick role.

These associations fit a hypothesis that the direction of effect is from maternal values to children's self-assessments (it would be logically less consistent to suggest that the direction be reversed: e.g., that because a child makes his own decision to adopt the sick role, his mother subsequently attaches importance to the need for self-direction by that child). That maternal values show relatively little association with _younger_ children's role appraisals may indicate that the process by which parental values impinge on children's role orientations is indirect, occurring during a relatively long time period. The older child may be more attuned to the subtleties of the communication process involved.

ROLE PREFERENCES AMONG JAPANESE WOMEN

Recent cross-cultural studies of psychiatric symptomatology by Carmi Schooler and his colleagues have found not only that female mental patients show more severe symptomatology than do male mental patients, but also that the general pattern of symptom differences found among cultures is reflected more strongly among women than among men. The importance of studying culturally defined sex roles in order to understand mental illness is emphasized by the results of research in the United States in which experimental, observational, and demographic studies showed that the relationship among symptoms is quite different among women than men. These studies indicate that the differences in symptom patterns between the sexes found among American patients may well have been strongly influenced by the effects of cultural definitions of sex roles.

These considerations prompted Schooler, in collaboration with Karen Smith of the Hyogo-Ken Mental Health Center in Kobe (now with the University of Pittsburgh), to undertake research on Japanese women's attitudes toward their roles. Japanese women's attitudes towards their various roles is particularly germane, not only because Japan was one of the countries included in the cross-cultural studies of symptomatology, but also because of the almost unique reputation of Japanese wives as exemplars of one type of sex-role definition. Nearly all literary descriptions of Japanese wives view them as paragons of domesticity who selflessly and subserviently meet all of their husbands' domestic needs without circumscribing their husbands freedoms. Needless to say, few of these descriptions come from the wives themselves.

This study asks Japanese wives about their own views on the subject, by means of a self-administered questionnaire, and relates their attitudes to their social characteristics. The most striking finding about the effects of social-structural variables on women's attitudes is the pervasive effect of social class. This is particularly noticeable for the respondent's evaluation of the importance of various roles. Higher social class directly results in a greater emphasis on the role of the individual, both for the respondent and her husband, and greater emphasis on the other-than-sex roles by the respondent. Thus, middle-class women who may, in comparison to working-class women, maintain some control over their destinies and who may be exposed to relatively complex environments, seem to place a greater emphasis on the role of the individual.

Social structural variables that increase personal autonomy and environmental complexity also appear to have a tradition-breaking effect on the interpersonal tone of the marriage. Higher present social class and urban upbringing, each independently increase the degree to which husband and wife socialize together and interact with each other as equals. A somewhat similar pattern can be seen in terms of the rejection of the traditional view of the characteristics of a good wife, such rejection being shown by the young, those with fathers employed in high-status occupations, and in relatively large-scale enterprises. Thus, in terms both of behavior and values, social-structural conditions that environmental complexity and individual autonomy lead to a breakdown of traditional hierarchical marital relationships.

Although at present Japanese women seem to accept severe restrictions on their life space without complaint, and to assume almost total responsibility for the household, there is some evidence of dissatisfaction and of desire for change. They wish their husbands to be more involved with the children. The traditional values of obedience and patience are now seen by Japanese women as relatively unimportant qualities for a good wife, and for many of them the role of person has become very important. Evidence of potential for change can also be found in the effects of the various social-structural variables. These findings support the hypothesis that social structural variables related to environmental complexity result in an increase in the emphasis on individuality and both greater equality and closeness in the husband-wife relationship. Certainly, if such personal attitudes and such characteristics of husband-wife relationships are related to environmental complexity, it seems reasonable to expect that they will become more prevalent as the complexity of life increases in Japan.

In any case, the sterotypic view of the Japanese wife as a paragon of selfless domesticity who is desirous of filling her husband's every wish probably needs revision. These data suggest that, although the Japanese wife's behavior may tend in this direction, psychologically she is more a disinterested than a willing servant. At present, her husband frequently does not seem to be a particularly important factor in the wife's social or psychological existence, being much less important to her than are her children. Certainly, when we actually ask Japanese women how important their various roles are to them, the order of the one chosen first is: Mother, person, and then . . . A Japanese Wife.

Annual Report for 1975

Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

Paul D. MacLean, M.D., Chief

As some analysts have observed, it is commonly assumed that experts arrive at economic, political, and other decisions on a purely "rational" basis. The same analysts emphasize that in the process there may be a failure to take into consideration unrecognized human "emotional" and "instinctual" factors that often heap wreckage on "rational" decisions. In this respect it is to be noted that human communicative behavior can be generally categorized as verbal and nonverbal. Many forms of human nonverbal behavior show a close parallel to behavioral patterns seen in animals. Since it is hardly appropriate to refer to nonverbal behavior of animals, it is desirable to use some such term as "prosematic," meaning rudimentary signaling, to apply to any kind of nonverbal signal -- vocal, bodily, chemical. One of the main purposes of this Laboratory, now in operation for four years, is to investigate brain mechanisms of species-typical, prosematic behavior. The relevance of such work on animals to human affairs becomes apparent when it is realized that the primate forebrain derives from three evolutionary developments that may be characterized as reptilian, paleomammalian, and neomammalian. Recent developments in histochemistry have helped to identify corresponding parts of the reptilian (striatal complex) and paleomammalian (limbic system) formations in reptiles, birds, and mammals.

The work of one section of the Laboratory focuses particularly on the functions of the striatal complex and limbic system in species-typical, prosematic behavior. In the continuation of a long-term investigation on brain mechanisms of the innate display of squirrel monkeys, it has been found that the striopallidal projections to the tegmental area are probably more essential for the display than those to the thalamus. These and other findings provide additional evidence that the two older evolutionary formations of the brain are fundamental for the expression of prosematic behavior.

In connection with this work there has been continued development of comparative studies on lizards. The significance of such research can be briefly summarized as follows: (1) As already mentioned, histochemical techniques have helped to identify corresponding structures of the forebrain in reptiles and mammals; (2) in the past year it has become evident that the pattern of organization of the three recognized aminergic systems of the brain in primates shows a striking similarity to that found in reptiles; (3) of existing reptiles, the behavior of lizards is believed to be most similar to that of the mammal-like reptiles which were the forerunners of mammals; (4) lizards and other reptiles engage in prototypical complex patterns of behavior commonly seen in mammals, including man.

Recognizing that research on lizards may help to give insights into the unknown functions of the striatal complex and the aminergic systems, the Administration encouraged our organizing a first-time Conference on the Behavior and Neurology of Lizards. The Smithsonian Institution and the National Zoological Park were cosponsors with us. After the first day of the meetings held here on May 5, the group of 30 scientists repaired to the Front Royal Research and Conservation Center for the remaining sessions on May 6 and 7. There was integrated for the first time such a wealth of diverse material on the behavior and neurology of lizards that the participants expressed the hope that the proceedings could be published.

With the introduction of hamsters to the Laboratory during the past year a new dimension has been added to the continuing work on brain mechanisms of olfaction. The sexual behavior patterns of male and female hamsters are unusually stereotyped and predictable. Mating behavior of the male hamster depends upon olfactory stimuli. Experiments in progress have shown that mating is still possible after the destruction of the olfactory bulbs provided that the vomeronasal nerves are left intact.

The quantification and analysis of behavior in the various studies are being facilitated by computer-assisted techniques developed by members of the Laboratory. Particular mention should be made of a computer-assisted, behavioral event recorder which allows large amounts of data to be acquired and reduced in short periods of time. A high-speed computer has also been utilized to generate structural chemical data for cholinesterase antagonists, as well as to analyze electrophysiological findings.

In the Section on Comparative Biopsychology there has continued to be work on the abstract problem of measuring and quantifying behavior. It is proposed that a particular behavior can be measured by "its capacity to fill time." The probability ($\underline{p}$) of a behavioral occurrence may then be expressed as the ratio of the time filled by that behavior to the total elapsed time. In arriving at this measurement there must be an estimate of $\underline{h}$, the time consumed during the occurrence of a particular behavior. Then the probability $\underline{p}$ is given by the equation $\underline{p} = \underline{ch}/\underline{t}$, where $\underline{c}$ is the number of occurrences and $\underline{t}$ is the total elapsed time. In the experimental work itself there has been a particular focus on adjunctive behavior, or what ethologists call displacement behavior. Such experimentation has clinical relevance insofar as it may give insights into the development of "nervous" habits. It was found in adult dogs that there was a moderate increase of adjunctive behavior when the operant conditions made it possible to imbibe alcohol.

In 1969, U-Thant made his famous pronouncement that there remained only 10 years to find solutions for the exploding population and related problems. The Section on Behavioral Systems continues to be deeply involved in such problems, having originally shown that the "psychological stresses" of crowding may bring about a collapse of social structure despite an ample provision of the necessities of life. The recognition of this possibility has far reaching implications as regards the mission of the National Institute of Mental Health. Since this section represents one of the few laboratories in our country doing work in this critical area, it is gratifying that there are plans for bringing here a group of six post-doctoral workers (with appointments of one to three years) to help the inauguration and implementation of a new series of studies scheduled for completion in about six years. One study will attempt to confirm previous findings that the pressures of crowding in a large population of mice eventually lead to disruptions of social behavior that are irreversible and end in the failure of procreation. A parallel study on rats will attempt to show that a computer-controlled provision of opportunities for cooperation among animals may prevent the sociopathology otherwise developing under crowded conditions.

Since this NIMH Laboratory represents a unique facility for conducting combined brain and behavioral studies under quasi-natural conditions, there would seem to be an obligation not only to engage in research, but also to provide experience for the growing number of young individuals expressing an interest in animal behavior, the influence of the environment on behavior, and the intermediary role of the brain. During the past year we have enjoyed the assistance of five volunteer guest workers from secondary schools and universities for periods of one to several months.

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health
Louis Sokoloff, Chief
July 1, 1974 through June 30, 1975

The Laboratory of Cerebral Metabolism during the last fiscal year has contracted from three Sections to two Sections by dissolution of the Section on Membrane Chemistry and incorporation of its only two remaining positions, both filled by technical support personnel, into other units of the Laboratory. This change was considered advisable because of dwindling research resources and major new research developments in the Laboratory Chief's own Section on Developmental Neurochemistry which necessitated allocation of greater resources to that research.

The Section on Membrane Chemistry was one of the three integral Sections comprising the Laboratory when it was first established in 1968 and was allocated adequate positions and budgetary support to sustain a creditable research effort in its area. A commitment was made to a senior investigator to appoint him Chief of the Section. This commitment was not accepted, and the Laboratory Chief, in addition to his position as Chief of the Section on Developmental Neurochemistry, also assumed the position of Acting Chief of the Section on Membrane Chemistry. In that capacity he directed the research activities of all professional personnel, supported either by NIMH or by outside grants, to whom commitments had been made earlier in the expectation that the Section would be a viable unit. The last professional personnel departed from this Section in June, 1974. Many of the positions originally allocated to the Section had been lost by administrative recapture or abolition of vacancies, and only two non-professional employees were left. It became clear that there were insufficient resources left from which to restore a viable Section, and despite the continuing importance of this area of research to a well-balanced neurochemical program, it was felt that there was little hope that the Section could be sustained except on paper.

In the meantime, a research idea with which the Laboratory Chief has intermittently concerned himself for the past 15 years abruptly crystallized within the last two years and led to the implementation of new research activities which have demanded as much as 75% of his time during the past year. It became clear that he could no longer devote the necessary time and effort to direct the research projects of the Section on Membrane Chemistry also, and inasmuch as it was impossible to recruit a senior investigator to be Chief of the vestigial Section on Membrane Chemistry, it seemed advisable to dissolve that Section's research projects and abolish it.

The other two Sections have continued their investigations in essentially the same projects as in the previous years with varying degrees of progress. These will be described below. It should be noted that the Laboratory has been fortunate in being able to obtain considerable help from guest workers and fellows supported by outside sources. Indeed, without such help, particularly in the new research directions referred to above, much of the progress would have been seriously impeded because of insufficient NIMH-budgeted personnel positions to meet the needs.

Section on Developmental Neurochemistry
Louis Sokoloff, Chief

This Section has continued its investigations in its four major areas o interest: a) the circulation and energy metabolism of the brain; b) the mechanism of action of thyroid hormones and their role in the postnatal maturation of the brain; c) the biochemistry of postnatal maturation of the brain; and d) the biochemical mechanisms of the alcohol-withdrawal syndrome.

a) Work in the area of the energy metabolism of the brain has resulted in spectacular progress during the last year. An idea first conceived by th Laboratory Chief approximately 15 years ago, when he was a Section Chief in the Laboratory of Clinical Science, has been conceptualized by him into a series of Laboratory experiments designed to develop a method for the measurement of the rates of glucose consumption in the discrete structural and functional units of the brain. Numerous theoretical and technical problems were encountered, and these obstacles have all been overcome in the last year. The method has been developed, it works extraordinarily well, an already exciting new knowledge of the brain is being acquired with it by us and by other laboratories trained in the use of the method by us. The basic principle of the method is the use of a radioactive analogue of glucose, $[^{14}C]$deoxyglucose, which is partially metabolized just like glucose but th stops and accumulates in the cerebral tissues in a quantitative relationsh to the rate of glucose consumption. On the basis of theoretical analyses, a equation has been derived which describes this relationship in measurable parameters from which local cerebral glucose consumption can be computed.

The method has been used to determine the rates of glucose consumption in the various functional and structural units of the brain in conscious and anesthetized rats. Those results not only establish the base-line normal values in the rat but also reveal the effects of anesthesia. Similar measurements are in progress in the cat and monkey. Studies with the method have demonstrated that normally local cerebral blood flow correlates very closely with cerebral energy metabolism. The method has also resulted in th definitive demonstration of what had been suspected but never proved, namely that, like in all other organs, energy metabolism is closely coupled to functional activity of the tissue within the brain as well. An exciting and potentially therapeutically useful finding has been the effects of α-butyrolactone, an agent alleged to block dopaminergic synapses in the brain. This agent has been found to produce a coma-like state during whic the cerebral energy metabolism is almost completely turned off, at least as much as is seen in profound hypothermia. The effect is reversible suggestin that the agent may turn out to be useful as a drug which can do everything for which hypothermia is presently used in neurosurgery and cardiac surgery without the complications and procedural complexity of hypothermia. This potentiality is under further study.

One of the features of this new method is the use of quantitative autoradiography to measure tissue concentrations of [^{14}C]deoxyglucose. These autoradiographs are pictorial representations of the relative rates of glucose consumption in the various structures of the brain. Therefore, even without quantitation the autoradiographs can be used to map functionally related neural structures and pathways simultaneously in the entire brain. Inasmuch as functional activity controls the energy metabolism, experimentally induced changes in functional activity can be used to trace all parts of the brain turned on or off. This is essentially an "evoked metabolic response" analogous to the classical evoked electrical potential used for years by neurophysiologists for mapping purposes. The advantage of the new method is that it maps the entire brain simultaneously rather than a few areas at a time. This method adds an enormously valuable new capability to the technology of neurophysiologists, neuroanatomists, neurologists, etc.

Despite the fact that very little has yet been published on this method, it has already received wide recognition throughout the world, and there has been a clamor from many laboratories to send personnel to us to aid in our experiments in return for learning the technique. We have accepted a number of these as guest workers, and they have been important, indeed essential, because the experimental demands of the development and applications of the procedure really exceed the personnel resources of our laboratory. We shall continue to pursue this modus operandi as far as possible.

b) Work on the mechanism of action of thyroid hormones has been continued mainly by Patricia Roberts, Elaine Kaufman, and Glenna Fitzgerald. Previous studies in this project had demonstrated that thyroid hormones stimulate protein synthesis, but that the effect is indirect and secondary to the formation of a product of a prior energy-dependent reaction between the hormone and some component of mitochondria. Studies have, therefore, been in progress directed at identifying the product and the nature of the biochemical reaction between the hormone and the mitochondria. An interesting new finding during the past year was that in the course of the reaction between mitochondria and the hormone, pyridine nucleotides are released from the mitochondria, and mitochondria from hyperthyroid animals have been found to have lowered contents of NAD^+. This effect could have very broad ramifications into all areas of metabolism because of the central role of the pyridine nucleotide in so many metabolic processes. This phenomenon is, therefore, under further study.

c) Elaine Kaufman, Glenna Fitzgerald, and J. D. Brown have continued studies of the postnatal development in brain of the enzymes of ketone body utilization. They have succeeded in solubilizing and partially purifying the first enzyme in the pathway of ketone body utilization, D-β-hydroxybutyrate dehydrogenase, from brain, but they have thus far been unsuccessful in their efforts to bring it to complete purity. In the meantime, however, they have been studying the possibility that this enzyme plays a role in Reye's Syndrome, a recently recognized condition in children, which sometimes follows viral infections and causes acute fatty degeneration of the liver, coma, and frequently death. A feature of this condition is that the fatty acid, octanoic acid, is elevated in the plasma. Our investigators have found that they can produce coma in rats by intraperitoneal injections of octanoic acid but not by intravenous injections, indicating that the octanoic acid must first be metabolized partially in liver for coma to occur. They have, in fact found that the injection of the octanoic acid leads to ketosis prior to the development of the coma. The octanoic acid has also been found to inhibit the D-β-hydroxybutyric acid dehydrogenase enzyme, suggesting the possibility that acetoacetate, formed from octanoic acid degradation in the liver, cannot be converted to D-β-hydroxybutyrate, and is itself the toxic agent in brain. This possibility is under further study.

d) R. J. S. Duncan has continued the project on the functions of alcohol dehydrogenase activity in brain and its relationship to the alcohol-withdrawal syndrome. Previous work in this project demonstrated the presence of such enzyme activity in brain, but its maximum activity in the brain as a whole is very small compared to that of liver and too small to affect appreciably ethanol metabolism within the brain. It is, however, a non-specific enzyme and its normal function could be in the metabolism of some other endogenous substrate which could be inhibited by the presence of alcohol. The definition of its function could be greatly facilitated by proper localization of the enzyme within the brain. Dr. Duncan has succeeded in purifying the liver alcohol dehydrogenase of rats and preparing rabbit antibodies against it which react with the brain enzyme as well. It is hoped to prepare fluorescent antibodies which could then be used with the aid of fluorescence microscopy of brain sections to localize the enzyme within the brain at the microscopic level.

Section on Myelin Chemistry

Marian W. Kies, Chief

The Section on Myelin Chemistry has completed a productive year with the following major contributions to basic science in the areas of neurochemistry and neuroimmunology:

1. Microheterogeneity of Myelin Basic Protein:

Two preparations of myelin basic protein (BP) differing markedly with respect to contents of different chromatographic forms have been studied in an attempt to elucidate the causes of their microheterogeneity. The analytical results showed that the five components differed sequentially by a single charge and the microheterogeneity resulted from secondary modifications (addition or removal of charged groups) of the most basic component (Component 1).

Two modifications were demonstrated (phosphorylation and loss of C-terminal arginine) and a third modification was postulated (deamidation).

From the data obtained on phosphorylation they were able to conclude that no more than two sites on the native polypeptide chain were phosphorylated in situ. Although their exact position in the chain has not been determined, one phosphate acceptor is a threonine residue and one is a serine residue.

2. Chemical Studies on CNS and PNS Basic Proteins:

Central myelin of most mammals contains a single basic protein of ∿ 18,000 M.W., comparable to the one they isolated and purified from guinea pig CNS myelin. Certain rodent species -- rats, mice, hamsters, etc., have a second basic protein in their central myelin similar to the first except for a deletion of approximately 25% of the polypeptide chain.

PNS myelin (specifically bovine and rabbit) contains two basic proteins, one of which appears to be almost identical to CNS-BP. The second PNS-BP is different in amino acid composition, conformation, electrophoretic behavior and immunogenicity from CNS-BP. Through cooperative studies with Dr. K. Uyemura of Saitama, Japan, they have extended significantly the published information on this second PNS-BP. They have shown that PNS-BP contains 2 cysteine residues (previously unreported), a C-terminal valine (previously reported incorrectly as lysine) and exists in solution almost entirely in the β-configuration (with very little unordered structure and essentially no α-helix).

PNS-BP is highly antigenic (i.e., the isolated protein induces antibody). However, it does not induce experimental allergic neuritis, which is readily induced by whole PNS myelin. Since the high content of β-structure is an indication of denaturation it is possible that the native conformation is required for the induction of this disease. None of the proteins isolated from PNS myelin has been found to be neuritogenic.

3. Prevention and Treatment of EAE: Identification of Specifically Active Regions in the BP molecule:

The preparation and characterization of specifically defined fragments of myelin basic protein have permitted them to identify the peptide regions in the BP molecule capable of inducing antibody by hyperimmunization as well as the peptide regions capable of interfering with development of EAE through preimmunization. A common peptide sequence appears to be required for all three immunologic reactions, i.e., induction or prevention of disease and antibody induction. Their hypothesis is based on the participation of specific T-cells in the total immune reaction of BP (T-cells which function as 1) effector cells in pathogenesis of EAE, 2) helper cells for induction of antibody-producing cells, and 3) suppressor cells).

In support of this hypothesis, they have found that (EAE resistant) Strain 2 guinea pigs fail to produce antibody by hyperimmunization (the mechanism for producing antibody which requires specific helper T-cells). However, they can produce antibody as readily as (EAE-susceptible) Strain 13 guinea pigs when this T-cell requirement is overcome by a large amount of mycobacteria in the BP-sensitization. With the extra mycobacteria, antibody is induced by all the BP fragments tested except peptide 1-20 (the amino terminal portion of the molecule).

Annual Report of the

Laboratory of General and Comparative Biochemistry

National Institute of Mental Health

Giulio L. Cantoni, Chief

In 1975, the Laboratory of General and Comparative Biochemistry enjoyed a reasonably peaceful and productive year. The severe budgetary restrictions, especially the limitation of budgetary positions, continued to affect adversely the overall productivity of the various members of the laboratory. While good work continues to be produced, many of the projects are inadequately staffed and supported and we continue to be unable to take advantage of the ideas, discoveries and new developments generated within the laboratory. Under these conditions, it is fair to say that the overall morale has continued to be adversely affected.

Drs. Cantoni, Uhlendorf and Scarpa continue to study muscle differentiation *in vitro*. The basic biochemistry of the differentiation process is of course very fundamental in the normal development of the various cell types of the brain and also of many other tissues. We have chosen as a model the growth and differentiation of embryonic skeletal muscle cells in culture. We have found that components of fetal calf serum are of critical importance in this switching mechanism; biochemical characterization of this material is under way. These approaches are in the mainstream of current efforts to understand the molecular biology of differentiation and the role of such substances as cyclic AMP and cyclic GMP in regulating these processes.

With Dr. Chiang and H. Richards, Dr. Cantoni continues his studies on the biochemistry of S-adenosylmethionine and S-adenosylhomocysteine. These investigations, now in their 25th year, continue to be extremely productive. S-Adenosylmethionine is a methyl group donor of central importance in human metabolism, functioning in some 80 different enzymatic reactions of intermediary metabolism. The mechanism of the two enzymes, S-adenosylmethionine synthetase and S-adenosylhomocysteine hydrolyase, has come under closer scrutiny and important new features of these important enzymes have been discovered.

Dr. Klee is studying the binding of morphine to specific membrane receptors in brain cells in tissue culture; this is one of the most important and interesting projects being carried out in this laboratory. His finding that cultures containing both neuroblastoma and glioma hybrid cells are richly endowed with morphine receptors whereas the parent neuroblastoma and glioma cells in culture are devoid of them, is of fundamental importance. Moreover, his finding that adenylate cyclase activity correlates with the number of morphine receptors is also very significant. Although many laboratories are currently working on receptors in narcotics in brain and nerve cells, Dr. Klee has developed a unique approach which has great promise.

Dr. Merril has continued his pioneer work on the transfer of a gene from bacterium into cultured human fibroblasts originally obtained from a patient suffering from galactosemia, a genetically determined disease of metabolism after association with mental retardation. He has found that the structural genes for the enzyme of galactose metabolism that are deficient in galactosemic patients can be introduced into human fibroblasts from bacteria and that they are able to express their genome and form the missing enzyme in the new environment. While the experiment is difficult to reproduce in 100 per cent of the cases, it has been repeated and reproduced both here and elsewhere. Dr. Merril is systematically investigating the various factors that are involved in the transfer and recombination of genes and characterizing the identification of the enzymes arising by transcription from the transplanted genes.

While there are many laboratories now working in the general area of gene transplantation, this whole area of research is of fundamental importance not only with respect to the mission of the NIMH, but also in approaches to all kinds of genetic deficiency diseases in humans.

Drs. Mudd, Giovanelli and Datko continue their important investigation on the biochemistry of sulfur containing amino acids in mammals and in plants. Inextricably involved in this project of the biochemistry of methyl transfer reaction that, as has been noted above, has been the principal preoccupation of the Chief of the Laboratory since its inception. These investigations, in keeping with the broad mission of the laboratory to investigate general and comparative biochemistry, may be divided into those studies of particular relevance to the mammalian biochemistry and those especially important for plant biochemistry. The distinction is somewhat artificial since better understanding of the metabolism of methionine and its biosynthetic pathway in plants has an important and practical bearing on several aspects of human nutrition.

During the past year, substantial progress has been made in both current projects in the Section on Alkaloid Biosynthesis. In the project on homocysteine biosynthesis in higher plants, a major effort was devoted to developing methods which would permit accurate determination of the extremely small metabolic pools of soluble cysteine, cystathionine, homocysteine, and methionine in Chlorella. Favorable conditions were developed for carrying out kinetic studies of the rates at which $^{35}SO_4^{=}$ labels these pools by building a chemostat and defining conditions of limiting sulfate. These methodological advances have been put to use to date in one experiment which promises to give a definitive assessment of the relative contributions of transsulfuration and direct sulfhydration to homocysteine biosynthesis under the specified conditions. It is expected, also, that the methods developed can be extended to other plants and to other nutritional conditions. Together, these studies should furnish the information for this area of metabolism which will be basic to most further studies.

For mammalian sulfur metabolism, the balance studies of labile methyl groups furnish first approximate answers to an interrelated series of questions concerning the relative importance of various transmethylations, the relative demands made by transmethylation and by polyamine synthesis, the extent of recycling of homocysteine, and the importance of preformed dietary, as compared to newly formed, methyl groups. Again, I feel that these answers furnish a framework needed for quantitative thinking about these areas of metabolism.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY

NATIONAL INSTITUTE OF MENTAL HEALTH

Ichiji Tasaki, Chief

The work of the Laboratory of Neurobiology has concentrated on the cellular basis of nerve function. Both the events related to nerve excitation and the events related to the communication between nerve cells have been studied.

Studies on nerve excitation centered on an analysis of the macromolecular organization of nerve membrane. The first approach to this problem was through (A) elucidation of the behavior of various dye molecules in the nerve membrane during the process of nerve excitation, and (B) analyses of the process of excitation by non-electrical means. Many dye molecules can be used to stain the nerve membrane and the optical properties (extinction coefficient, absorption and fluorescence emission spectra, quantum yield of fluorescence, molecular orientation, etc.) of the dye molecules in the membrane can be determined. This laboratory had previously shown that the conformational changes in the membrane macromolecules during nerve excitation can be studied by examining the optical properties of the dye molecules. This year we were able to incorporate two kinds of dye molecules in the nerve membrane of squid axons or of crab nerves and to study resonance transfer of electronic energy between the molecules. Furthermore, by measuring the dye absorbance during nerve excitation, it was found that transient changes in the state of aggregation of a certain class of dyes can be examined.

The following results were obtained during the past year: We examined the optical properties of 8 different positional isomers of amino-naphthalene-sulfonate in the nerve membrane, and found that the sensitivity of the optical properties to the solvent polarity is essential for production of changes in fluorescence intensity during nerve excitation by a dye molecule. The phenomenon known as resonance transfer of electronic energy was demonstrated by incorporating p-chloro-anilino-naphthalene-sulfonate (donor) and Eastman merocyanin-540 (acceptor) in the nerve membrane. The average distance between these donor and acceptor molecules in the membrane was estimated. Changes in the absorption spectra of a variety of dye molecules in nerve membrane were demonstrated during nerve excitation. These findings indicate that either the state of aggregation or the absorption spectrum of the dye molecules undergo transient changes when the membrane is electrically excited. Under intracellular perfusion, it was found possible to excite the nerve membrane by various non-electric means. Changes in the internal hydrostatic pressure, osmolarity of the perfusion fluid, internal pH, etc., were found to be effective means of exciting the nerve membrane. The relationship between electrical and non-electrical means of exciting the nerve membrane was found.

To understand the behavior of the nervous system under normal and pathological conditions, it is essential to clarify the process of nerve excitation on the molecular basis. There is little doubt that the effects of many chemicals which affect the function of the nervous system are brought about by binding of the chemicals with the nerve membrane. Since excitable nerve membranes are extremely labile, it is usually very difficult to apply standard biochemical methods to study the mode of non-covalent binding of chemicals to the nerve membrane. The results of the optical and electrophysiological studies described above have already elucidated important aspects of the labile structure of the nerve membrane and its changes during nerve excitation.

Another approach to the study of nerve excitation was to isolate and characterize the membrane macromolecules which undergo conformational change during nerve excitation. Proteins with molecular weights of 120,000, 68,000, and 14,000 were found to incorporate I^{125} strongly. Some I^{125} incorporation is also found in the peptide with a molecular weight of 46,000. It is known that iodine reacts only with tyrosine and histidine residues of the proteins. There are few labeled compounds available which do not pass the cell surface. Therefore, these labeled proteins are located on the surface of the nerve membrane. These biochemical and biophysical techniques have the potentiality for pointing out the specific molecules that are participating in the process of nerve excitation.

The communication between cells and the relation of cells to behavior was studied in the visual system of the primate. To do these experiments, we studied the discharge of single cells in the brain of awake monkeys trained to do a number of visual-motor tasks. We have studied the visual pathways at three different anatomical points: the superior colliculus, the lateral geniculate nucleus, and the circumstriate cortex.

In the first area studied, the colliculus, we wanted to see whether visual cells might contribute to the distinction man makes between stimulus movement in the external world and stimulus movement produced by his own eye movement We found that 60% of the cells in the superficial layers of the colliculus responded to stimulus movement across the stationary receptive field of cells over a wide variety of range of stimulus velocities; it was clear these cells could discharge a stimuli swept in front of the eye at velocities comparable to those occurring during an eye movement. We then studied such cells to determine their response to stimulus movement produced by the eye movement sweeping the receptive field across a stationary stimulus. About half of these cells did not respond to stimulus movement caused by eye movements; these cells are therefore able to distinguish between stimulus movements resulting from eye movements and those resulting from real world stimulus movements. It seems unlikely that visual factors prevent these cells from responding with eye movements since these cells continue to distinguish eye induced from real stimulus movement when the same experiments are conducted in a darkened environment where visual factors are greatly reduced, and since these cells show a suppression of background firing whenever the monkey makes spontaneous eye movements in total darkness. Thus these cells probably receive a non-visual signal about the occurrence of an eye movement, and it is this signal which prevents them from responding to stimulus movements caused by eye movements. No such distinction of stimulus movement is made by striate cortex neurons so that these cells in the colliculus perform a function frequently postulated but never found in the primate.

The second area investigated was the lateral geniculate nucleus and the experiments were designed to determine whether any influence of the eye movement on visual processing occurred after the eye movement. The response to a visual stimulus was measured during successive fixation trials and compared to the response when the stimulus was presented 0, 100, 200 and 500 msec after completion of saccades from another part of the visual field to the fixation spot. The visual response of 40% of the neurons tested changed when the visual stimulus was presented after a saccade and the effect is usually maximal during the first 200 msec after the saccade. On cells which showed a dramatic change in response following a saccade across a lighted background, there is no change in the cell's response following a saccade across a darkened background. This modulation of visual processing therefore results from the sweep of the visual stimulus across the retina rather than from any extra-retinal input. In a few cells there is a consistent change in the visual response following a saccade across a darkened background, suggesting that some modulation of visual processing may result from an extra-retinal source.

The third area studied was the circumstriate area of cerebral cortex just anterior to the termination of the pathway to striate cortex. Cells in the buried posterior bank of the lunate sulcus were studied, and about 90% of all cells were visually driven. Most of these cells fall into distinct groups. Oriented cells are best driven by a properly oriented and positioned slit of light. Spot-pandirectional cells respond best to small spots of light anywhere over a relatively large area of the visual field. Directional cells are especially concerned with moving stimuli; they respond best to a stimulus moving in a certain direction at an optimal velocity. Inhibited-by-light cells have remarkably high spontaneous activity and are inhibited by a large spot filling the receptive field. Color cells do not have much orientation preference, but are, on the whole, concerned with the color of the stimulus falling on their fields. Side-stopped cells have receptive fields that may be mapped with small spots to show an excitatory region. These experiments confirm the expectation that the visual processing begun in striate cortex proceeds in circumstriate cortex. The processing of different stimulus features is clearly divided between cells, some are more responsible for color some for orientation, etc. Thus a parallel processing of different stimulus features by different cell groups emerges as the apparent mode of operation of this area of cortex.

Annual Report of the Laboratory of Neurochemistry

National Institute of Mental Health

Seymour Kaufman, Chief

For the last 15 years, one of the major problems of the Laboratory of Neurochemistry has been the analysis of the complex hepatic enzyme system that converts phenylalanine to tyrosine.

There have been many reasons for studying this enzyme system, but perhaps the most compelling one is that it seemed to offer the possibility of providing insight into the pathophysiology of the disease, phenylketonuria (PKU). And, of the dozens of inborn errors of metabolism, PKU appeared to hold special promise for helping us to understand the normal development and functioning of the brain. As Jervis, one of the pioneers of research in PKU, has said, "The study of phenylpyruvic amentia may throw light on the whole problem of mental deficiency....".

Our main approach to the problem of understanding the phenylalanine hydroxylating system has been an attempt to dissect the system into its individual components. To date, two enzymes, the hydroxylase and dihydropteridine reductase, and two coenzymes, reduced pyridine nucleotide and tetrahydrobiopterin, have been shown to be essential. Indeed, the discovery of the last compound in this Laboratory was a logical consequence of the approach that had been adopted. In addition to these four components, two others - PHS and dihydrofolate reductase - have been shown to play key roles in the hydroxylation reaction.

As a result of the successful analysis of this system, we have been able to unequivocally identify the hydroxylase as the missing component in the classical form of PKU. These efforts have been described in previous reports.

One of the bonuses earned from the identification of the individual components of the hydroxylating system is that this knowledge provides a rational basis for predicting the occurrence, and, indeed, some of the clinical symptoms, of variants of PKU that might be due to a lack of a component other than the hydroxylase, itself. We have previously suggested that variants lacking either the pterin cofactor or dihydropteridine reductase might be encountered in practice. Since work in the Laboratory of Neurochemistry has shown that both of these compounds are also essential components of the hydroxylases responsible for the biosynthesis of the neurotransmitters, dopamine, norepinephrine and serotonin, it could be anticipated that the lack of either of these components would lead to defects in the metabolism of neurotransmitters.

During the last year, in collaboration with a group of clinicians at Johns Hopkins School of Medicine, we have identified and been studying the first patient with a form of PKU caused by a deficiency of dihydropteridine reductase. The patient, a male infant, not only has signs of impaired phenylalanine hydroxylation, but also suffers from neurological deterioration even on a low phenylalanine diet. Using specific and sensitive enzyme assays developed in the Laboratory of Neurochemistry, we have shown that the patient's hepatic phenylalanine hydroxylase levels may be somewhat decreased, but the decrease is not marked enough to account for his symptoms. By contrast, he has no detectable dihydropteridine reductase activity in any of his tissues that have been examined - liver, brain, and cultured skin fibroblasts, whereas the corresponding tissues from control patients have high activity of this enzyme.

We have prepared a specific antiserum to pure sheep liver dihydropteridine reductase and have confirmed, with immuno-diffusio techniques, that the reductase is affected in this patient's liver.

Studies of his neurotransmitter metabolism indicate that just as anticipated, the lack of reductase does lead to defects in the rates of synthesis of dopamine and serotonin.

Although it is possible that this patient's brain has already been irreversibly damaged (he is now about 1 1/2 years of age), we are currently evaluating the therapeutic effectiveness of administration to him of the metabolic products beyond the enzyme defect, i.e., 5-hydroxytryptophan and DOPA. It is too early to tell whether this treatment is effective in releiving his neurological symptoms.

We have previously found that in the classical form of PKU, there is some phenylalanine hydroxylase activity. This finding has stimulated studies of mechanisms for enhancing the activity of this enzyme. Specifically, we have focused on mechanisms that might be applicable to regulating the activity of the enzyme in humans. We have found that the activity of the purified rat liver hydroxylase can be increased by subjecting it to phosphorylation. Phosphorylation was effected by a cyclic-AMP-dependent protein kinase. Although the increase in activity after phosphorylation is only two-fold, we have found that the native rat liver enzyme is already partially phosphorylated. These results raise the possibility that the completely dephosphorylated enzyme might be devoid of hydroxylase activity. Based on our findings, a comparison of the state of phosphorylation of the enzyme in PKU liver with that of normal liver becomes of great interest. The unavailability of fresh human liver, however, makes it unlikely that this comparison can be made in the near future.

We have previously shown that tyrosine hydroxylase shares many regulatory properties with phenylalanine hydroxylase. Our finding that the latter enzyme could be activated by phosphorylation, together with the evidence that tyrosine hydroxylase could be activated by polyanions, strongly suggested that tyrosine hydroxylase could also be activated by phosphorylation.

Using highly purified preparations of brain tyrosine hydroxylase, we have shown that the enzyme can be markedly activated by a protein kinase-dependent process. Activation leads to a pronounced decrease in the K_m value for the pterin cofactor. We are currently trying to determine whether the hydroxylase itself, is phosphorylated during the activation process.

During the last year, we have succeeded in purifying tyrosine hydroxylase from brain to a state of near homogeneity. We expect that the availability of the essentially pure enzyme will facilitate these studies on the mechanism of activation of the enzyme in phosphorylation.

Significant progress has been made in analyzing the system which is capable of the *in vitro* production of integrative recombinant-DNA. The initial characterization of the reaction has shown that it is the *in vitro* counterpart of the naturally-occurring *in vivo* recombination. This conclusion is based on the following lines of evidence: 1) the system depends on the presence of the same phage-coded gene products as are required *in vivo*; 2) the product DNA formed *in vitro* has the same genetic makeup and physical length as that formed following infection of whole cells. In addition to the characterization of the DNA product, progress has been made in separating and purifying the individual components of the *in vitro* system, including a product of the *int* gene and two host-coded components. This system continues to hold the promise for increasing our understanding, not only of viral integration, but also of genetic recombination in many systems of biological and medical interest.

In the area of cell membranes, it has been found that the surface membrane insulin receptor of the insulin-resistant obese mouse, which was previously shown to be reduced four-fold in concentration, is functionally normal in terms of equilibrium and kinetic constants, biological specificity, and cooperative interactions. This finding strengthens the previous hypothesis that receptor concentration is regulated by control mechanisms which allow the cell to adjust its response to outside signals.

Annual Report of the Laboratory of Neurophysiology

National Institute of Mental Health

Edward V. Evarts, M.D., Chief

July 1, 1974 -- June 30, 1975

This summary of the research program of the Laboratory of Neurophysiology will describe three areas of ongoing research according to which the projects of the Laboratory may be categorized. Each of these areas has been subdivided and the summaries will be organized according to these major areas and their subdivisions.

I. Brain Mechanisms in Motor Control

1. The role of cerebral motor cortex in control of movement
2. Eye-head coordination in the primate: neural substrate and plasticity
3. The activity of red nucleus neurons in movement

II. Metabolic Correlates of Activity in Brain and Muscle

1. Neurochemical and neuroanatomical correlates of behavior
2. Muscle function and metabolism

III. Functional Neuroanatomy

1. Anatomical and physiological analysis of thalamic input to the primate motor cortex
2. Transport mechanisms across membranes and the blood-brain barrier

Before these areas are summarized, however, some highlights of the findings in each of the three areas will be listed and the significance of these findings in relation to the research goals of the Mental Health Institute will be pointed out.

In studies on Brain Mechanisms in Motor Control it has been found that nerve cell activity in the motor control area of cerebral cortex reflects the intention of the subject as to the direction of a forthcoming movement rather than merely reflecting the motor act itself, as would be the case for the muscle. This finding provides important new information concerning the brain mechanisms which correspond to the psychological processes of "set" and "readiness," and begins to bridge the gap between central psychological variables and overt behavior. As such, this finding contributes to an understanding of the cerebral mechanisms underlying higher mental processes.

In addition to discovering certain central correlates of intention and "set," our studies of movement have made important contributions to an understanding of the processes which underlie the plasticity of behavior. It is plasticity which allows modifications of behavior as a result of experience, and any insights obtained into brain mechanisms underlying plasticity aid in the understanding of complex mental processes such as learning and memory.

Our current work on eye movements is particularly concerned with the mechanisms involved in plasticity of the reflex control of visually guided behavior. Prominent in this work is the hypothesis that the cerebellum may be important in changes of reflex gain as a result of experience. Our studies have demonstrated that the plasticity of the vestibulo-ocular reflex of the Rhesus monkey is adaptive. In these experiments monkeys were fitted with telescopic spectacles which either magnified the world by a factor of two or diminished it to half of its normal size. The vestibulo-ocular reflex of monkeys fitted with such spectacles gradually underwent adaptations such that the magnitude of eye movements generated in association with the monkeys' head movements became appropriate to the compensatory eye movement needed to maintain stable retinal images during head movements. The role of the vestibulo-ocular reflex is to maintain a steady retinal image during head turns, and the adaptive plasticity in these experiments helped the reflex to accomplish this purpose.

In work on Metabolic Correlates of Brain Activity, the second major phase of the work of the Laboratory of Neurophysiology, it has been shown that localized regions of the brain become selectively active in certain sorts of behavior. These selectively active areas can be pinpointed by utilization of a technique which reflects their localized metabolic activity. Utilization of this technique has led to a series of rather basic discoveries over the last two years in LNP and the Laboratory of Cerebral Metabolism, with which we have been collaborating. First it was found that stimulation of the sciatic nerve in the anesthetized rat increased the glucose consumption in the ipsilateral dorsal horn of the spinal cord several times the control side. It was next found that enucleation of the eye of a rat resulted in decreases of metabolism in the structures related to vision. Later it was found that swimming rats showed large but rather generalized increases of metabolism as compared to resting rats. This was the first demonstration that a physiological change could result in an increase of metabolism.

Improved resolution with tritiated 2-deoxyglucose showed that the glucose consumption of neuronal perikarya was only slightly greater than the surrounding neuropil and that the bulk of gray matter glucose consumption is accounted for by neuropil. This indicated 2-deoxyglucose may be useful in mapping changes of neuropil metabolism related to function.

Rotation of rats results in increased metabolism in the vestibular nuclei and vestibulocerebellum. This demonstrated localized changes of metabolism related to a physiological stimulus. Exposure of rats to specific odors results in characteristic localized increases of metabolism in the olfactory bulb. This demonstrated a spatial representation of odor in the olfactory bulb which will undoubtedly provide the basis for a long series of studies.

The results described above show how this project is already opening new areas of investigation and providing new information concerning the brain. The methods used will be adopted widely to redefine and continue to explore the function and anatomy of the nervous system. It is hoped that the techniques being developed could provide additional tools to study behavioral, pathological, and pharmacological states of the central nervous system. The project has already benefited greatly from the collaboration of the Laboratories of Neurophysiology and Cerebral Metabolism and should provide eventual ties with Neuropharmacology and Neuropathology within the Institute.

The third major research area of the LNP deals with Functional Neuroanatomy. Work in this area seeks to clarify the functional significance of the thalamus, a region which links the cerebral cortex with major subcortical systems. Thus, the outputs of the basal ganglia and the cerebellum converge on the ventrolateral nucleus of the thalamus (VL) which is the major source of subcortical input to the motor cortex. The thalamus can play an important role in the initiation and control of movement by processing and relaying the outputs of the major subcortical motor nuclei to the motor cortex. Analyses of the function of the thalamus in movement control have previously relied largely on the techniques of stimulation and ablation, or cell recording in anesthetized animals. Our current work is unique in that it applies both anatomical and physiological techniques to discover the origin of thalamic inputs to the motor cortex, and to learn more about the function of the thalamus during normal movement.

Experimental and clinico-pathologic studies in both animals and man have long indicated that the basal ganglia and cerebellum play an important role in the control of movement and posture. Abnormalities in movement, posture, and involuntary movements frequently result from damage to these structures produced either experimentally or through disease.

Tha anatomical studies in this project utilize the phenomenon of "retrograde axonal transport" to label the origin of inputs to the motor cortex. Low molecular weight proteins injected into the motor cortex are "taken up" (through a relatively unknown process) by synaptic terminals in the motor cortex. These proteins are then transported from the terminals, through the axon, back to their cell body (i.e., retrograde axonal transport). When the tissue is processed by the appropriate histochemical methods, the protein is visualized in the somas and proximal dendrites of neurons which send their axons to the motor cortex.

The results of our combined anatomical and physiological experiments reveal that neurons related to active arm movements are found within the same ventral thalamic region which the anatomical studies identified as the VL arm area. Units related to jaw, tongue, and neck activity associated with the juice reward occurred only medial to the VL arm area. Units related to spontaneous leg movements occurred only lateral to the VL arm area.

It has been suggested that in a number of movement disorders, the thalamus plays a prominent role in propagating the abnormal signals of diseased subcortical structures to the motor cortex. For this and other reasons, VL thalamic lesions have been made in human patients for a number of years (e.g., to reduce the tremor and rigidity accompanying Parkinson's disease). The discovery, in this project, of a previously unknown source of thalamic input to the motor cortex (from the intralaminar nuclei) should cause many of the theories on the anatomical basis of movement disorders to be modified. Thus, the present project should provide a firmer basis for the treatment of brain disorders causing abnormal movements by furthering our understanding of the role of the thalamus in the control of normal and abnormal movement. Patients with motor disorders such as parkinsonism and Huntington's disease exhibit psychological disturbances as well as movement abnormalities, and information in these illnesses contributes to the research goals of the Mental Health Institute by clarifying the neural basis for these psychological disturbances.

I. BRAIN MECHANISMS IN MOTOR CONTROL

1. The Role of Cerebral Motor Cortex in Control of Movement

In primates, a zone of cerebral cortex lying anterior to the central fissure (precentral motor cortex) contains a set of neurons whose axons pass without interruption to the spinal cord via a nerve bundle named the pyramidal tract; the neurons of this tract are called pyramidal tract neurons (PTNs). In the spinal cord the axon terminals of PTNs end both in the interneuronal pool and on alpha and gamma motoneurons.

In considering the functional role of motor cortex PTNs, it is useful to compare PTN activity with the activity of spinal cord α-motoneurons (α-motoneurons innervate skeletal muscle, whereas γ-motoneurons control the sensitivity of intramuscular stretch receptors). Studies in the Laboratory of Neurophysiology have utilized microelectrode recording techniques to investigate how motor cortex PTNs control movement. In contrast to α-motoneurons, which are inactive during muscular inactivity, most PTNs are tonically active even during total muscular quiescence. Furthermore, recordings of PTN activity in awake monkeys make it clear that discharge frequencies of PTNs are being modulated and controlled even in the absence of any movement. We have recently carried out studies of PTN discharge in monkeys who are able to carry out differing movements depending upon the "instructions" they have received. These studies have revealed that one factor influencing motor cortex PTN discharge frequency in the absence of overt muscular contraction is the intention to move. The variation in PTN activity in relation to intention to move is seen even before any muscular activity occurs. Thus, when a monkey has learned that a particular signal (e.g., a red light) means he should flex his elbow, PTNs adopt a pattern of discharge corresponding to the intention to flex. A different pattern of PTN discharge frequency is adopted if the monkey receives a signal (e.g., a green light) instructing him to extend. These patterns of PTN activity specific to the impending movement occur well in advance of any overt muscular contraction, for in these experiments the monkeys were required to wait several seconds between the instruction and a second signal which told them that the time had come to carry out the instruction. Thus, motor cortex neurons show specific patterns of activity depending not only on a movement which is in progress but also depending upon a movement which is impending.

In the experiment referred to above, the "instruction" (a red or green light) was followed by a second stimulus, which was a signal to the monkey to carry out the movement. This second stimulus was a perturbation of the handle grasped by the monkey, and such a stimulus activates receptors which send information both to the motor cortex and to the spinal cord. A second striking feature of motor cortex PTN activity which differentiates it from the activity of spinal cord motoneurons is the extent to which the responsiveness of PTNs to such sensory inputs may be modified depending upon the movement which this sensory input will trigger. Thus, the responsiveness of PTNs to incoming sensory stimuli was "gated" on or off depending upon the instruction which the monkey had received, and thus, depending on the movement he intended to make. This gating of PTN responsiveness to sensory inputs allows selectivity of motor behavior depending upon decisions made by the subject. The motor cortex may be thought of as a flexibly controlled center in which input-output relations can be changed.

2. Eye-head Coordination in the Primate: Neural Substrate and Plasticity

The preceding section of this Summary dealt largely with studies on parts of the central nervous system which play a role in the control of limb movements. Another major segment of recent work on motor control in LNP dealt with control of eye movements. The models which investigators have sought to explore in examining the activity of central neurons in association with eye movement have been based on the notion that there are four oculomotor sybsystems controlling saccadic, smooth pursuit, vestibular, and vergence eye movements. Almost all eye movements in primates are combinations of the movements produced by each of these subsystems.

Our current work on these subsystems controlling eye movement is particularly concerned with the mechanisms involved in plasticity of reflex control. Prominent in this work is the hypothesis that the cerebellum may be important in changes of reflex gain as a result of experience. Studies by Miles have demonstrated that the plasticity of the vestibulo-ocular reflex of the Rhesus monkey is adaptive. In these experiments monkeys were fitted with telescopic spectacles which either magnified the world by a factor of 2 or diminished it to half of its normal size. The vestibulo-ocular reflex of monkeys fitted with such spectacles gradually underwent adaptations such that the magnitude of eye movements generated in association with the monkeys' head movements became appropriate to the compensatory eye movement needed to maintain stable retinal images during head movements. The role of the vestibulo-ocular reflex is to maintain a steady retinal image during head turns, and the adaptive plasticity in Miles' experiments helped the reflex to accomplish this purpose.

In addition to studying the plasticity of the vestibulo-ocular reflex, Miles has studied single unit firing patterns in vestibular nuclei related to voluntary eye movements and passive body rotation in monkeys. The results of these experiments demonstrate that individual neurons in the vestibular nuclei are points of convergence between impulses representing central eye movement programs and impulses bringing feedback information from the periphery. This convergence between the two sorts of information underlies the automatic eye-head coordination which is a feature of the eye motor control system. This system of automatic eye-head coordination provides compensation for the magnitude of the head movements which ordinarily accompany saccadic eye movements.

Additional studies by Miles have involved recordings of single cells from a portion of the cerebellum known as the flocculus. These studies have revealed that the Purkinje cell output from this area discharges specifically in relation to visual tracking towards the ipsilateral side. These cells generate a velocity profile of the pursuit target which, within limits, is largely independent of any passively superimposed head rotations during tracking. Most of the mossy fiber input elements discharge in relation to saccadic eye movements or labyrinthine inputs, but we have identified a class of visual units responding to retinal image slip in the region of the fovea with strong preferences for ipsilateral motion. Such units may provide the basic visual information from which the Purkinje cell fashions a velocity construct of the track target.

The results of recordings in the flocculus suggest that, in the primate at least, this structure is involved in smooth pursuit eye movements and may indeed provide oculomotor centers with the target velocity information needed to support such tracking. These ideas are in line with very recent clinical findings which point to deficits in smooth pursuit eye movements when this part of the cerebellum is compromised.

3. The Activity of Red Nucleus Neurons in Movement

The use of single cell recording techniques in association with movement is allowing studies which will lead to an understanding of the functional role of structures which until now have been almost total mysteries. One such structure is the red nucleus. In studies on the red nucleus, monkeys were trained to depress a hold key for a period of 2 sec. After this hold period either a red or a green cue lamp appeared in a random sequence. The red lamp required the monkey to move his hand from the hold key to a target button within 1 sec of the cue presentation. The green lamp required continued depression of the hold key for 1 sec following presentation of the cue. Correct performance was reinforced with fruit juice. Firing of red nucleus (RN) units was found to be related to movements of particular body parts, e.g., upper and lower limbs, face (mandible, tongue, eyelid), eyes and neck. In addition, some units showed modulation of firing after the green light presentation. In comparing post-stimulus and peri-response latencies of 48 RN and 46 motor cortex (MC) units related to the arm movement triggered by the red lamp, the onset of activity in most RN units occurred after the onset of activity in most MC neurons. For 48 RN neurons, the peak of the distribution of onset times was shifted 80 msec later than for 46 MC neurons. This delay between MC and RN is even greater than the delay between MC and postcentral gyrus. It is known that RN receives powerful inputs both from the sensorimotor cortex and from cerebellum--and it seems possible that the activity in RN was dependent on the combined action of these two inputs, with sensory feedback from movement (relayed via postcentral gyrus and/or cerebellum) being one input and a central program from cerebellum and/or precentral gyrus being the other input.

II. METABOLIC CORRELATES OF ACTIVITY IN BRAIN AND MUSCLE

1. Neurochemical and Neuroanatomical Correlates of Behavior

Within recent years advances in neuroanatomical techniques have made it possible to examine the role of brain cells in ways that promise rapid progress in our understanding of brain and behavior. Our work in this area ia aimed at obtaining neurochemical and neuroanatomical correlates of central nervous system activity occurring in association with behavior. Such information could provide valuable correlates for the microelectrode techniques in use in the Laboratory. Microelectrode techniques are limited because they selectively record activity from a small set of large cells. Ideally, chemical changes of smaller neurons, neuronal processes, and glia should eventually be related to a behavioral state.

To implement the above objectives we have used an autoradiographic technique in which radioactive deozyglucose is an indicator of glucose utilization of localized cerebral regions. Behavioral changes are correlated to regional changes in glucose uptake. This shows anatomical localization of metabolically active areas and cells, interrelationships between active and inactive areas, and provides basic biochemical data on cerebral metabolism.

Utilization of this technique has led to a series of rather basic discoveries over the last two years in LNP and the Laboratory of Cerebral Metabolism, with which we have been collaborating. First it was found that stimulation of the sciatic nerve in the anesthetized rat increased the glucose consumption in the ipsilateral dorsal horn of the spinal cord several times the control side. It was next found that enucleation of the eye of a rat resulted in decreases of metabolism in the structures related to vision. Later it was found that swimming rats showed large but rather generalized increases of metabolism as compared to resting rats. This was the first demonstration that a physiological change could result in an increase of metabolism.

Improved resolution with ^{3}H 2-deoxyglucose showed that the glucose consumption of neuronal perikarya was only slightly greater than the surrounding neuropil and that the bulk of gray matter glucose consumption is accounted for by neuropil. This indicated 2-deoxyglucose may be useful in mapping changes of neuropil metabolism related to function.

Rotation of rats results in increased metabolism in the vestibular nuclei and vestibulocerebellum. This demonstrated localized changes of metabolism related to a physiological stimulus. Exposure of rats to specific odors results in characteristic localized increases of metabolism in the olfactory bulb. This demonstrated a spatial representation of odor in the olfactory bulb which will undoubtedly provide the basis for a long series of studies.

The results described above show how this project is already opening new areas of investigation and providing new information concerning the brain. The methods used will be adopted widely to redefine and continue to explore the function and anatomy of the nervous system. It is hoped that the techniques being developed could provide additional tools to study behavioral, pathological, and pharmacological states of the central nervous system. The project has already benefited greatly from the collaboration of the Laboratories of Neurophysiology and Cerebral Metabolism and should provide eventual ties with Neuropharmacology and Neuropathology within the Institute.

2. Muscle Function and Metabolism

The relations in muscle among metabolism, regulation of ionic homeostasis, and contraction are not understood clearly. The interaction among these fundamental mechanisms is important to the comprehension of basic muscle physiology and the more complicated analysis of muscle disease and disorders of movement.

When a whole striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue does not occur in dystrophic muscles, and is characteristic of fast but not slow mammalian and amphibian muscle fibers. The complexity of the excitation-contraction process in fast muscles makes it difficult to determine where and when fatigue sets in. In order to simplify this complex process for experimental analysis, single muscle fibers of the frog semitendinosus muscle were selected to study fatigue and

and recovery, rather than whole muscle, to avoid rate-limited diffusion and the heterogenous fiber population of whole muscle.

If the muscle fiber is stimulated tetanically in Ringer made hypertonic by the addition of sucrose, contraction is abolished, although the heat of activation and calcium release and uptake are not. When the fiber is returned to isotonic Ringer solution, twitch tension is normal and has not declined. The prevention of fatigue by hypertonic Ringer indicates that repetition of a step in the contraction process causes fatigue, probably by reducing local concentrations of ATP and creatine phosphate which are necessary to support contraction. These high energy metabolites probably are not synthesized rapidly enough from available glycogen or from lactic acid to prevent fatigue in muscles tetanized in normal Ringer solution.

These results define the parameters of stimulation and the time course of fatigue and recovery in single fibers, and make it possible to correlate individual points in the course with the exact spectrum of metabolites--ATP, creatine phosphate, glycogen--in the fibers. Metabolic analysis of single fibers has not been employed before because measured quantities are at the range of 10^{-12} moles, but in collaboration with Dr. Janet Passonneau we intend to use them for an exact correlation of the fatigue-recovery process and the energy state of the single fiber.

The relation between function (contraction, calcium release and uptake, electrical activity) of a single fiber and the energy state of the muscle is important not only to understanding the basic mechanism of excitation-contraction coupling and fatigue and recovery of muscle, but to see how metabolites are employed generally to support cellular activity. The understanding of these mechanisms in normal muscle should help to comprehend defective relations in diseased muscle.

III. FUNCTIONAL NEUROANATOMY

1. Anatomical and Physiological Analysis of Inputs to the Primate Motor Cortex

The outputs of the basal ganglia and the cerebellum converge on the ventrolateral nucleus of the thalamus (VL) which is the major source of subcortical input to the motor cortex. Thus, the thalamus can play an important role in the initiation and control of movement by processing and relaying the outputs of the major subcortical motor nuclei to the motor cortex. Analyses of the function of the thalamus in movement control have previously relied largely on the techniques of stimulation and ablation, or cell recording in anesthetized animals. Our current work is unique in that it applies both anatomical and physiological techniques to discover the origin of thalamus inputs to the motor cortex, and to learn more about the function of the thalamus during normal movement.

Experimental and clinico-pathologic studies in both animals and man have long indicated that the basal ganglia and cerebellum play an important role in the control of movement and posture. Abnormalities in movement, posture, and involuntary movements frequently result from damage to these structures produced either experimentally or through disease.

It has been suggested that in a number of movement disorders the thalamus plays a prominent role in propagating the abnormal signals of diseased subcortical structures to the motor cortex. For this and other reasons, VL thalamic lesions have been made in human patients for a number of years (e.g., to reduce the tremor and rigidity accompanying Parkinson's disease). The discovery, in this project, of a previously unknown source of thalamic input to the motor cortex (from the intralaminar nuclei) should cause many of the theories on the anatomical basis of movement disorders to be modified. Thus, the present project should provide a firmer basis for the treatment of brain disorders causing abnormal movements by furthering our understanding of the role of the thalamus in the control of normal and abnormal movement.

Tha anatomical studies in this project utilize the phenomenon of "retrograde axonal transport" to label the origin of inputs to the motor cortex. Low molecular weight proteins injected into the motor cortex are "taken up" (through a relatively unknown process) by synaptic terminals in the motor cortex. These proteins are then transported from the terminals, through the axon, back to their cell body (i.e., retrograde axonal transport). When the tissue is processed by the appropriate histochemical methods, the protein is visualized in the somas and proximal dendrites of neurons which send their axons to the motor cortex.

The results of these physiological experiments support the concept of a VL arm area developed from the anatomical studies. Neurons related to largely active arm movements were found within the same ventral thalamic region which the anatomical studies identified as the VL arm area. Units related to jaw, tongue, and neck activity associated with the juice reward occurred only medial to the VL arm area. Units related to spontaneous leg movements occurred only lateral to the VL arm area.

A comparison of VL neuron and muscle activity indicates that some VL neurons modify their activity as much as 60 msec before any changes in muscle activity. This finding is consistent with the hypothesis that some VL neurons play a role in movement initiation.

2. Transport Mechanisms Across Membranes and the Blood-Brain Barrier

The action of drugs and hormones on the brain depends in large measure on their entrance into nervous tissue from the blood, and this passage involves crossing a system referred to as the "blood-brain barrier." For several years, a major research effort in our Laboratory has sought to understand the nature and function of the blood-brain barrier, and to amplify the theory of active and passive transport across cellular membranes. Research on the brain and pharmacotherapy of the brain and meninges often are hindered by the restrictive influence of the barrier in preventing drugs from entering the brain. It would be useful, therefore, to be able to modify barrier permeability, reversibly and without affecting neuronal function, in order to overcome these restrictions.

The aim of this project is (a) to understand the basic morphological and physiological properties leading to and protecting barrier function and integrity; (b) to develop a method to open the barrier, based on these properties, that may prove useful in brain pharmacotherapy; and (c) to understand how the barrier responds to different stresses to the brain, including the loss of autoregulation of cerebral blood flow.

Different methods are employed in these studies. Surgical procedures are used to open the skull and dura of rabbit, rat, or Rhesus monkey, and to isolate and perfuse the internal carotid artery. Neurological examinations are performed to evaluate function, and methods of perfusion and fixation are used for brain and eye histology. Quantitative chemical techniques are employed for analysis of wet and dry weights of brain tissue, and atomic absorption spectrophotometry employed for studying brain electrolytes.

Section on Neurochemistry
H. Weil-Malherbe, Chief

Report from the Research Group of Dr. H. Weil-Malherbe

Ammonia Formation in Brain

The mechanism of ammonia formation in the central nervous system continues to be of principal interest in our laboratory. In the period under review work has focused on two areas: the effects of hadacidin, a specific inhibitor of adenylosuccinate synthetase, and the subcellular distribution of brain adenylate deaminase. Both adenylosuccinate synthetase and adenylate deaminase may be involved in ammonia formation as members of the "purine nucleotide cycle" group of enzymes.

(a) The effect of hadacidin (H. Weil-Malherbe). Hadacidin inhibited the ammonia formation of respiring brain cortex slices in a substrate-free medium although it proved to be less potent than other inhibitors previously studied, such as 5-bromofuroate, a specific inhibitor of glutamate dehydrogenase. At low concentrations, the effects of these two inhibitors were additive, suggesting that hadacidin and 5-bromofuroate specifically affected two different mechanisms. However this conclusion is valid only if additivity persists at concentrations which are approaching saturation. At high concentrations the effects of the more potent inhibitor, 5 bromofuroate, were still enhanced by the addition of hadacidin but in decreasing degrees.

A kinetic analysis of the inhibitory effects showed a significant difference between the maximum inhibition obtainable by hadacidin and 5-bromofuroate; moreover, the two inhibitors had a higher affinity for the target enzyme systems when used in combination than when used singly, suggesting a potentiating effect of the combination.

To obtain further insight into the mode of action of hadacidin we plan to study its effects on the concentration of endogenous free amino acids in brain slices.

(b) Studies on adenylate deaminase (H. Weil-Malherbe)

An actomyosin-like protein has recently been described in brain synaptosomes by Berl et al. (1973). These authors named it neurostenin and suggested that it is involved in the process of transmitter discharge by exocytosis. As it is well-known that myosin of muscular origin has adenylate deaminase activity even after extensive purification, it seemed of interest to establish whether this also applies to neurostenin. At the same time, the intracellular distribution of adenylate deaminase in brain tissue, a subject on which widely divergent results have been published, was studied.

As anticipated, neurostenin, twice reprecipitated, had adenylate deaminase activity which was 3-4 times as high as that of any of the other subcellular protein fractions. Nevertheless, of the total enzyme content in the brain, only about 2% was associated with neurostenin. About 34% of the total activity was found in the cytoplasmic fraction, 42% in the mitochondrial fraction, 15% in the synaptosomal fraction and 7% in the microsomal fraction.

Indole Metabolism By Brain Slices

(a) Formation of tryptamine and serotonin from L-tryptophan (H. Weil-Malherbe)

Although L-tryptophan is a substrate of aromatic amino acid decarboxylase in brain, its affinity to the enzyme is low, and the K_m-value is much above the physiological concentration of tryptophan in the whole brain. It is not surprising, therefore, that several authors failed to observe a formation of tryptamine by brain slices. As tryptamine may, to some extent, pass through the blood-brain barrier, the question arises whether the tryptamine present in the brain actually originates there. The question is important in view of the possible function of tryptamine as a neurotransmitter and of the hypothesis that it is involved in the etiology of schizophrenia.

In our experiments slices from various areas of guinea pig brain were incubated with physiological concentrations (2×10^{-5} M) of ^{14}C-labeled L-tryptophan in the presence of 5×10^{-5} M pargyline. The amines formed were isolated by cation exchange chromatography and separated by thin layer chromatography. An unequivocal formation of tryptamine was demonstrated in the four brain areas tested: hypothalamus, corpus striatum, the median part of pons and medulla, and cerebral cortex. The rate of tryptamine formation was highest in the hypothalamus (0.63 nmol/g/h) and significantly different from the rate in the other three areas where about 0.2 nmol/g/h were formed.

Serotonin formation was highest in hypothalamus and pons-medulla (about 3.2 nmol/g/h) and lowest in corpus striatum (0.96 nmol/g/h).

Attempts were made to study the kinetics of the reaction with slices of cerebral cortex as these were the only ones available in sufficient quantity. The apparent K_m -value for serotonin formation corresponded to a tryptophan concentration of about $4\text{-}5 \times 10^{-5}$ M. The formation of tryptamine however was practically linear with increasing concentrations of tryptophan up to 3×10^{-4} M, the highest concentration tested and it was therefore not possible to compute a value for K_m, except that it is likely to be $> 10^{-4}$ M.

The difference in the kinetics of serotonin and tryptamine formation in cerebral cortex slices resulted in a decrease of the ratio of serotonin to tryptamine formation with increasing concentrations of L-tryptophan until the rate of tryptamine formation equalled or exceeded the rate of serotonin formation.

The differences in the rate of tryptamine formation and in the ratio of tryptamine to serotonin formation observed in different areas of the brain at a constant concentration of L-tryptophan may be due to differences of K_m. This point is under investigation.

(b) Serotonin metabolism in brain slices (J. W. Gordon)

The in vitro metabolism of serotonin by slices of different areas of brain has not been studied extensively by other investigators, and where it has been studied usually only the major metabolite, 5-hydroxyindole acetic acid (5-HIAA), has been determined. We have developed methods for the separate estimation of radioactive 5-HIAA, 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole acetaldehyde (5-HIALD).

The three metabolites were found to be formed in the same proportion by slices of the four brain areas studied, hypothalamus, corpus striatum, pons and cortex, namely in the ratio of 2:10:100 for 5-HIALD, 5-HTOL and 5-HIAA, respectively. Slices of corpus striatum, pons and cortex had very similar activities and metabolized about 25 nmoles/g/h when incubated with 3.6×10^{-5} M serotonin. Hypothalamus, however, had roughly twice this activity.

It is planned to study the effect of various agents and conditions on the metabolism of serotonin. So far, the effect of substrate depletion has been examined. The ratio of the metabolites was not affected by this experiment but the overall rate of metabolism was reduced by about 50%, indicating perhaps a reduced uptake of serotonin (a process known to be dependent on the supply of energy). The unchanged ratio of metabolites suggests that the experiment did not lead to any major breakdown of co-enzymes.

Particular attention was paid to the detection of minor or unknown metabolites . None have been found so far. An unidentified radioactive derivative of serotonin, at first thought to be a new metabolite (see Annual Report for 1974), eventually turned out to be the methyl ester of 5-HIAA formed when the mixture of metabolites was passed over a column of Dowex 50 and eluted, at a weakly acid reaction, with 60% methanol. When the Dowex 50 column was replaced by a column of Amberlite CG-50 and 5-HIAA was washed out with water, the formation of the methyl ester was no longer observed.

Formation of L -α- hydroxyglutarate from α-ketoglutarate in tissue slices

Despite various modifications in methodology it has not been possible to overcome the difficulties inherent in the instability of the starting material and to obtain statistically valid results. The project has been abandoned.

Miscellaneous

At the invitation of Professor Essman, the editor of a handbook on "Serotonin in Health and Disease", H. Weil-Malherbe wrote a review article on "Serotonin and Schizophrenia". H. Weil-Malherbe also acted as an External Examiner and Thesis Referee for J. J. Warsh, a Ph.D. Candidate at the Institute of Medical Science, University of Toronto. He is currently supervising the work of J. W. Gordon who is a Ph.D. candidate at the Department of Biochemistry, George Washington University Medical School, Washington, D.C.

LABORATORY OF NEUROPHARMACOLOGY

Floyd E. Bloom, M.D., Chief

This summary document is the seventh report in the history of the Laboratory, and in anticipation of my resignation in the Fall of 1975, will be the last such report which I shall fully prepare. Recognizing the changes in direction and leadership which must be determined in the next several weeks in order to maintain the scientific coordination and momentum of the present Laboratory, I have, for this final report, asked the Section Chiefs to characterize their own accomplishments and indicate their own plans for the next phases of the research they have chosen to pursue. While the Laboratory has in fact achieved many considerable scientific accomplishments in the past several years, and has been particularly productive this year, I am personally and especially pleased that the Laboratory has also been so fortunate as to have produced these three senior independent scientists: Drs. Hoffer, Siggins and Weight, with whom I have been privileged to work.

In preparing an informative summary of our year's activities, the abundance of scientific data and concepts which have been produced provide an embarrassment of riches so vast that to dwell on any one would provide an exciting story. I have, therefore, extended this introductory statement slightly, in order to provide some overall perspective on our activities which will be helpful in fitting together the more detailed descriptions of each of the Sections. Close readers of past reports will recognize at the outset that a characteristic pattern of our activities becomes lost in a Section-by-Section style description, namely the strong collaborative, interdisciplinary nature of our work. These studies permitted us to detect and characterize several monoaminergic projections within the central nervous systems of several mammalian species, which have provided the scientific methods and foundations upon which all of the very exciting work described below was built, and from which highly substantive new projects are now evolving.

With respect to our most fundamental scientific mission, the characterization of the norepinephrine and dopamine mediated projections has permitted the evaluation of psychotherapeutic agents at sites known to transmit by these chemicals. Phenothiazines, widely considered to act in schizophrenia therapy exclusively as anti-dopamine agents, were shown to affect several aspects of norepinephrine transmission as well. Lithium, known to stabilize the affective swings of manic-depressives, was shown to have a selective antagonistic effect on norepinephrine synaptic inhibition in the hippocampus. The actions of amphetamine at the cellular level were again demonstrated to be post-synaptic in both dopamine and norepinephrine target areas.

At the molecular level of research, our work has shown that norepinephrine and dopamine mediated central synapses can be intimately associated with the production of cyclic adenosine monophosphate, and that cyclic AMP may mediate transmitter effects within the cell, presumably by activating a specific protein kinase. Cyclic AMP iontophoretically applied within hippocampal pyramidal neurons is effective in altering membrane properties, while catecholamine transmitter substances work only on the extracellular surface; furthermore, derivatives of cyclic AMP inhibit the firing of cerebellar target cells in direct correlation to their ability to activate brain protein kinases. In attempting to develop a histochemical method to localize adenylate cyclase in potential target neurons, it was discovered that lead, in extremely low concentrations, inhibits the enzyme; this effect could underlie some of the cerebral signs of lead poisoning. Finally, other molecular work reported this past year from our group indicates that in cerebral cortex and sympathetic ganglia cyclic GMP may mediate intracellular control mechanisms which oppose those mediated by cyclic AMP.

At the cellular level, new methods have been evolved for the detection of monoamines by light microscopy and to trace their circuits. Methods have been developed to examine the growth and development of the central nervous system regions which give rise to or receive monoamine pathways, and to determine in normal or genetically defective animals the effects and possible roles of monoamines in these areas with respect to experimental variables such as undernutrition. Applying such methods, it has been possible to examine the pharmacological and physiological properties of neurons maturing in brain explants cultured in vitro or transplanted in vivo into the anterior chamber of the eye of a host animal. The culture work has resulted in the first known instance of longterm maintenance of catecholamine production in vitro for several weeks, and will be an obviously exploitable preparation for both neurobiology and neuropharmacology. The ability to detect the retrograde movement of axonal particles in vitro reveals yet another unsuspected major property of all living nerve cells, the continuous particulate progression of chemical material from periphery to central soma. In expressing the ability of neurons to develop outside of the central nervous system, to organize and remain viable, and to be attracted to the precise and exclusive target cell as in situ, the norepinephrine terminals of the sympathetic nervous system present a remarkable insight into the almost magical biology of brain development. Aside from these fundamental possible insights into cell surface markers, synapse formation and function, the in oculo transplants have also provided us with a model system for determining precise dose-response curves for interesting drugs on neuronal discharge which is possible through the superfusion of drug solutions through the anterior chamber.

At the behavioral level, our recent work has begun to reveal the means by which the synaptic projections arising from the monoamine cell body regions can in fact regulate the performance of the whole animal. The norepinephrine source neurons in the nucleus locus coeruleus have been shown to be directly re-inforcing on rat behavior. Rats quickly learn to self-stimulate their locus coeruleus to receive the "reward" of an electrical stimulation there. The precise activation of this internal reward mechanism in conjunction with other sensory events detected at the neural level, and combined with the molecular effects possible through the activation of protein kinases, suggest that the monoamine pathways which we have been studying could underlie the longer-term changes which are more simply termed memory, and that these pathways could also be influential in attributing to any sensory event the re-inforcing or aversive quality by which much of everyday social interaction and vegetative function is regulated.

SECTION ON COMPARATIVE NEUROBIOLOGY
George R. Siggins, Ph.D., Acting Chief

The Section on Comparative Pharmacology is primarily concerned with study of the role of monoamines, and especially norepinephrine, in vertebrate neurotransmission, using model monoaminergic systems from the nervous systems of various species. We seek to answer the following questions: 1) how do monoamine-containing neurons synthesize, store and transport their monoamines to their axonal terminals; 2) what mechanisms are triggered when the released monoamine interacts with the postsynaptic target cell. To address these problems, we currently employ the following comparative test systems arranged in decreasing order of organizational complexity: 1) The intact cerebellum of the normal, anesthetized rat; 2) the cerebellum of the anesthetized normal and weaver mutant mouse; the cerebellum of the latter is genetically devoid of parallel fibers and granule cells; 3) chronic primary cultures or explants of central and peripheral mammalian and amphibian neurons; 4) acutely isolated (teased) axons from the frog sciatic nerve.

THE POST-SYNAPTIC ACTION OF CATECHOLAMINES: INVOLVEMENT OF OF CYCLIC AMP AND PROTEIN KINASE

It has become increasingly apparent from previous studies on rat cerebellum caudate, hippocampus and cerebral cortex (see Project Reports: Z01 MH 01184, 01223, 01224, 01257, 01298, 01301, and 01302) that the potent inhibitory action of norepinephrine and dopamine in these structures is mediated intracellularly by cyclic AMP, which may be generated by the interaction of the catecholamine receptor with a membrane bound adenylate cyclase. This hypothesis grew out of studies showing that cyclic AMP applied by iontophoresis from 5-barrel micropipettes to cerebellar Purkinje cells generally mimics the inhibitions produced by exogenous norepinephrine or by activation of the adrenergic input to Purkinje cells from the brain stem nucleus locus coeruleus (LC). All three of these manipulations produce hyperpolarization of rat Purkinje cells with a unique increase in input or membrane resistance. However, it is conceivable that this effect in intact cerebellum could evolve from indirect, dysfacilatatory effects of the drugs or LC stimulation on a tonically-active, excitatory pathway from granule cell/parallel fibers to Purkinje cells. To eliminate this possible source of indirect effects of drugs and LC stimulation on Purkinje cells, we have this year studied Purkinje cells of the weaver mouse. In this mutant, Purkinje cells lack granule cell inputs, as almost all granule cells die; however the mutant Purkinje cells still receive a rich adrenergic input from the LC and retain almost all their postsynaptic thickenings (and, therefore, probably transmitter receptors).

Drs. Henriksen, Landis and I found that Purkinje cells of the weaver mouse are capable of sustaining a near-normal spontaneous discharge of action potentials, in spite of the deficit of the major excitatory input to the cell. Moreover, stimulation of the LC and iontophoresis of norepinephrine and cyclic AMP produces inhibitions of the discharge apparently identical to those seen in normal mouse and rat cerebellum. Indeed, most of the neurotransmitters and drugs tested by iontophoresis (serotonin, gamma aminobutyrate, glutamate, norepinephrine, cyclic AMP, cyclic GMP) had identical effects in the weaver compared to normal. Only acetylcholine, which was mostly excitatory in weaver and inhibitory in normal mouse, produced different effects. Thus, the data support a direct effect of most iontophoretically applied drugs and of LC stimulation on Purkinje cell activity.

However, the question still remains as to how the cyclic AMP itself generated post-synaptically by release of catecholamine, could trigger the hyperpolarization with increased membrane resistance (decreased ionic conductance). A hint to this question may be found in the suggestion by Paul Greengard and his colleagues that protein kinases may be universally involved in the diverse physiological actions of cyclic AMP. Thus, intracellular cyclic AMP might activate protein kinase which in turn would phosphorylate a protein (presumably a membrane constituent) which might then alter membrane conductance to (or pumping of) some ion such as sodium. To test this possibility physiologically, Dr. Henriksen and I applied various anologues of cyclic AMP (possessing differing degrees of potential for activating brain protein kinase) to rat cerebellar Purkinje cells by iontophoresis. The results indicate that cyclic AMP analogues depress Purkinje cell discharge in direct and significant correlation with their ability to stimulate brain protein kinase in vitro, thus implicating protein kinase in the inhibitory action of cyclic AMP and norepinephrine.

Further analysis of the biophysical action of cyclic AMP and protein kinase awaits the development of our tissue culture methodology. Drs. Shoemaker, Forman and Schlumpf have succeeded in growing excitable central and peripheral neurons in culture, with the object of recording membrane properties by intracellular techniques during application of drugs and replacement of the bathing medium with fluids having altered ionic composition. I have written computer programs (for PDP-12 computer) to automatically generate and store current/voltage curves from intracellular stimulation of neurons. Drs. Padjen, Forman and I have chosen for this electrophysiological study the large frog sympathetic ganglion neurons in culture. Current/voltage curves from these neurons give a detailed picture of the specific membrane resistance and capacitance of the neurons and how these properties change with application of certain neurotransmitters such as gamma-aminobutyrate (GABA) and acetylcholine. We now have evidence that the unique depolarizing effect of GABA is a result of an increased conductance to chloride ions. However, to date it has proved difficult to evoke reproducible responses of these frog neurons to catecholamines or cyclic AMP, probably due to technical difficulties in delivering sufficient quantities of drug to the cell. Our current approach is to inject cyclic AMP directly into the cell by pipette or other means.

SYNTHESIS, STORAGE AND RELEASE OF CATECHOLAMINES

Drs. Shoemaker and Schlumpf have adapted the catecholamine-radioassay technique of Axelrod to neurons in tissue culture, with a view toward co-culturing catecholamine-containing cells with appropriate target neurons. This technique has then been applied to cultures of sympathetic ganglia and brain nuclei (locus coeruleus, substantia nigra) known to synthesize and store catecholamines in vivo. Their studies with various culture media show that the brain catecholamine nuclei, when cultured as explants, still are capable of synthesizing catecholamines, but cannot store them in significant quantities. Dr. Forman verified this finding by showing lack of catecholamine-induced fluorescence in the cells, using the Falck-Hillarp histochemical method for catecholamines. The catecholamines are instead found in large quantities in the culture medium. Addition to the growth medium of nerve growth factor, various catecholamine-enzyme co-factors such as ascorbic acid, copper ions, and DMPH4, or metabolic poisons of the rapidly dividing non-neural cells, had virtually no effect on this catecholamine leakage or on the net synthesis. Co-culturing of substantia nigra with caudate (the target tissue) or LC with cerebellum also was without effect. Furthermore, culturing brain nuclei in culture medium optimum for growth of sympathetic ganglia leads to cell death. Conversely, incubation of sympathetic ganglia with medium optimal for brain cells, even with added nerve growth factor, also leads to cell death. It therefore appears that some unknown factor or condition present in vivo is required for central catecholamine neurons to retain their transmitter in vitro, and that major differences in the media required for survival exist between catecholamine-synthesizing cells in brain and ganglia. Studies are now in progress to elucidate these unknown factors or conditions.

AXONAL TRANSPLANT

Axonal transport is a fundamental process of all neuron types, and is the process whereby chemical communication is possible between the neuron soma and its distant terminals. It is known that active axonal transport carries the enzymes necessary for synthesis of catecholamines and other neurotransmitters to the synapse, and is responsible as well for bringing back these and other chemical messages from the periphery.

A promising new way to study axonal transport is the direct observation, by specialized light microscopy, of the movements of subcellular particles. Drs. Forman, Padjen and I have filmed these movements, by time-lapse cinemicrography, in carefully dissected axons from bullfrog sciatic nerve and in cultured neurites of frog dorsal root ganglia and rat cerebellum. Frame-by-frame measurements of the particle movements are then performed with an L-W motion analyzer system and analyzed in numerous ways by a PDP-12 computer, providing the most extensive quantitative study ever made of such particle movements.

The basic features of the organelle movements so far clarified are: 1) the movements occur at an average velocity of about 90 mm/day, consistent with the fast mode of axonal transport characterized by tracer studies; 2) the movements are saltatory and bidirectional; 3) the velocities of individual particles vary from moment to moment and net speeds vary; however, velocity distributions of the entire population of particles in a fiber are similar from axon to axon; 4) most (90%) of the visible particles move toward the cell body; 5) particle movement is temperature sensitive, with a Q of 3. Low temperatures block the movements as do certain drugs such as chlorpromazine.

Thus, it appears that analysis of particle movements represents a promising new way to study axonal transport, yielding information on basic mechanisms of transport not accessible to approach by the more popular radiotracer and enzyme accumulation methods. Future plans are to study the effect of various neuroactive drugs on the particle movements, to identify what the particles are, and to perform sophisticated statistical analysis of the movements in an effort to delineate the possible mechanisms underlying the transport.

SECTION ON CYTOCHEMICAL PHARMACOLOGY

Floyd E. Bloom, M.D., Chief

The Section on Cytochemical Pharmacology has continued experiments designed to characterize and identify the cytological location of particular synaptic terminals in terms of their transmitter content and post-synaptic receptor mechanisms. As in the past, major emphasis has been placed on identifying--by light and electron microscopy--the site and destiny of norepinephrine-containing nerve terminals on target cells to be studied by electrophysiological and pharmacological methods in other Sections, and to attempt to complement those studies by testing the involvement of cyclic nucleotides at these synaptic sites. Notable highlights of the past year's activities have been the development of a new light microscopic method of great speed and improved sensitivity for the identification of catecholamine-containing neurons and nerve terminals, and the combination of behavioral observations with cytochemistry on the functional role of norepinephrine fiber systems in unrestrained rats and squirrel monkeys.

Ms. Battenberg collaborated with Dr. Bloom to develop a new procedure for the demonstration of catecholamine-containing fibers and neurons by fluorescence microscopy by modifying the glyoxylic acid methods of Lindwall and Bjorklund to the cryostat. The resultant procedure makes possible the rapid demonstration of even the most fine fiber systems within minutes after the conclusion of an experiment. Thus far, the new method has been used to document the natural occurrence of norepinephrine fibers in the cerebellum of normal rats and mice, neonatal rats, x-irradiated rats, cerebella and hippocampi transplanted in oculo and several types of hypocerebellar mutant mice. In addition, Dr. Koda has initiated experiments designed to determine whether the improved sensitivity of the glyoxylic acid "fixation" can be combined with electron microscopy to improve the resolution and detection of catecholamine fibers at that level.

Ms. Battenberg and Dr. Bloom have applied the immunocytochemical method for the localization of bound cyclic nucleotides in two collaborative studies. With Drs. John Kebabian and Paul Greengard of Yale University, we have shown that slices of bovine sympathetic ganglia incubated in vitro with dopamine show increased cyclic AMP bound within the post-ganglionic neurons with no effects on cyclic GMP. Under the same conditions, the same class of cells shows increased binding of cyclic GMP when the slices are exposed to acetylcholine. This cytochemical evidence supports the proposition that the cyclic nucleotides, cyclic AMP and cyclic GMP, are able to mediate opposing cellular functions for separate lines of chemical synaptic transmission on the same class of target nerve cells, and thus extends the possibilities of this opposing dualism as developed from electrophysiological tests of the rat cerebral cortical pyramidal tract neurons (Stone, Taylor and Bloom, 1975).

In collaboration with Drs. D. Goodman and H. Rasmussen of the University of Pennsylvania, Dr. Bloom and Ms. Battenberg have shown that epithelial cells of the toad bladder react to the hormone oxytocin by greatly enhanced binding of cyclic AMP, in a dose-response and timed response sequence.

Both these studies serve to confirm the interpretation that the immunocytochemical localization of cyclic nucleotides is capable of reflecting tissue cyclic nucleotide content by a method which is cell-specific and nucleotide selective.

In attempts to extend the cytochemical characterization of cyclic nucleotide involvement in synaptic transmission, Dr. Nathanson has pursued studies which were designed to provide a valid cytochemical method for the localization of neurotransmitter-sensitive adenylate cyclase. Previously published methods for such a procedure did not deal with nervous tissue, and preliminary experiments by Dr. Bloom and Ms. Battenberg had suggested that methods based on the precipitation of lead phosphate could reveal norepinephrine catalyzed enzyme activity. To verify this suggestion, Dr. Nathanson implemented a cyclic AMP assay and an adenylate cyclase activity to determine the effects of the cytochemical reaction reagents on the enzyme and substrates, and to determine the optimal reaction conditions for the cytochemical reaction. Surprisingly, it was learned through these experiments that lead would inhibit the enzyme at extremely low levels and also cause the non-enzymatic catalysis of the substrate; thus, in the presence of Pb, the enzyme is inactive, but the substrate will break down, forming a visible reaction precipitate. Presumably this precipitate has been the endpoint previously reported, but is undoubtedly plagued with so much artefact as to be uninterpretable. While the search for a useable method is still underway, Dr. Nathanson has been able to formulate experiments which suggest that behavioral dysfunctions of lead toxicity in man could in fact result from this action of lead on adenylate cyclase. In recent experiments, Drs. Nathanson and Shoemaker have measured the changes in hormonal sensitivity of brain regions to neurotransmitters in terms of adenylate cyclase activity. These experiments will test the possibility that specific forms of nutritional deprivation (in this case postnatal protein deprivation) alter brain development by selective depression of certain forms of chemical transmission. If either norepinephrine or dopamine activated adenylate cyclase are altered by this perturbation, then the adenylate cyclase assay system should reveal changes in the dose-response peaks indicative of modified sensitivity. If successful, such experiments could establish usefulness of the dose-response peak points of the enzyme assay as an index to the degree of function of the synapses in vivo.

Drs. Shoemaker and Bloom and Ms. Battenberg have continued to explore means of acquiring a computer-based optical analysis method for the more rapid quantification and morphometric characterization of synaptic contact points based on the ethanolic phosphotungstic acid method. In this year, renewed efforts have been made to collaborate with DCRT at NIH. Thanks to the support of Dr. Judith Prewitt, this project again seems to have some opportunity of success. Efforts are now underway to utilize the optical analyzing systems available at the Jet Propulsion Laboratories of Cal Tech, supported by grants from DCRT, to analyze our material from normal and undernourished rats. If successful, these methods will establish another morphological index to be used in the characterization of the deficits attendant to undernutrition and its recovery upon re-feeding. In addition, this method could be used to assess the phenomena of axonal sprouting and to determine the changes in synapses with aging, as well as to characterize the changes in specific morphometrically characterized synaptic populations.

In other experiments, Drs. Koda and Henriksen are investigating the ultrastructural changes in nerve terminals and perikarya throughout the 5-HT containing neurons of animals given two drugs which produce biochemical changes: p-Cl-amphetamine and fenfluramine. In addition to providing some experimental tools for the selective elimination of central serotonin neurons, these experiments may also be expected to reveal whether the appetite suppressant drug fenfluramine is able to produce nerve cell death in animals. If evidence suggests that it can, consideration must be given to notification of the FDA of this apparently unnecessary side effect of an appetite suppressant.

Unit on Psychobiology: In order to begin to develop methods which would reveal the behavioral significance of the neuronal circuits which were capable of detection by cytochemical methods, a unit on psychobiology was established. In the past year three types of experimentation were performed, all of which have successfully yielded exciting new data.

In rats, Dr. Segal has shown that electrodes placed in the locus coeruleus will elicit self-stimulation behavior in rats. When the rats are also implanted with fine wires to detect unit activity in the hippocampus (to which the locus coeruleus projects); each activation of the self-stimulation electrode will produce inhibition of the neuronal discharge in the hippocampus. Administration of the drugs alpha-methyl tyrosine, diethyldithiocarbamate or 6-hydroxydopamine will block both actions (self-stimulation and unit activity depression), while amphetamine facilitates the effects on both actions. When the locus coeruleus is co-activated with auditory or visual signals to indicate food reward, the animal learns more quickly to press the lever. When evaluated in terms of the effects on hippocampal unit discharge, the conditioning experiments indicate that the stimulation of the norepinephrine circuits increases the responsiveness of the hippocampal units to the conditioning signal.

Also in rats, Dr. Robinson has developed an experimental model to examine the psychobiological sequelae of cerebro-vascular lesions. In rats, as in man, small cortical strokes lead to brief paraplegia and more prolonged periods of agitated depression. In the rat model, these changes are associated with increased locomotor activity during a 5-7 day period when cortical norepinephrine levels are biochemically depressed. Application of the new glyoxylic acid method indicates that cortical fibers can recover during the next ten day period as the major behavioral changes recede. If animals are treated throughout the post-stroke period with desmethylimipramine, an effective human antidepressant, the animals behavior remains normal and the fluorescent properties of the locus coeruleus neurons and cortical fiber projections appears to be enhanced. These studies suggest that some of the behavioral sequelae of the stroke-model lesion may represent the hypofunction of cortical norepinephrine fibers while the recovery from these deficits may indicate nerve sprouting.

In the squirrel monkey, Drs. Foote and Freedman have documented the existence of a norepinephrine-containing projection from the locus coeruleus to the auditory cortex and have pursued in the awake monkey the influence of iontophoretically applied norepinephrine on responses of cortical units to squirrel monkey vocalizations. These studies also indicate that while norepinephrine is itself depressant on spontaneous cell activity, during the application of norepinephrine, responsive cortical units increase the effects of a particular vocalization. In subsequent experiments with Dr. Schwartz, Dr. Foote has pursued this preparation by attempting to record neurons from the locus coeruleus and to determine on these units the effects of the vocalization known to affect units in the auditory cortex. To facilitate the acquisition of heuristic data, monkeys in these experiments are also trained beforehand to respond to visual signals by bar pressing for liquid rewards. If brain circuits arising from the locus coeruleus are responsive to such reward-revealing experimental manipulations, we expect that direct observation of the activity of the locus coeruleus neurons will reveal this relationship more clearly. However, two problems have arisen which have considerably slowed progress on this project: the stereotaxic approach to the squirrel monkey is quite lengthy and minute errors in the blind navigational approach have so far outweighed successful hits. To improve accuracy, we are developing x-ray based triangulation techniques and the extension of guiding tubes for the microelectrodes. In each case, cytological information is needed to verify the units from which we recorded during the experiments. As a result each monkey can be used only for a limited number of recording sessions before adjacent electrode tracts become obscured or eliminated by the healing process. Therefore, this experiments requires the frequent replenishment of experimental monkeys and the current embargo on exportation of central and south american squirrel monkeys threatens the supply of sufficient experimental subjects. We have attempted to breed our own, but in the laboratory setting with the limited space we have to devote to such attempts, progress will be too slow to provide ample material. Unless some direct exchange with Bolivia or Peru becomes possible, we will have to consider replacing the squirrel monkey with some other small, non-infectious, easy-to-use, primate and if this is required, considerable fundamental anatomy and physiology will have to be repeated. Hopefully this expensive repetition can be avoided.

SECTION ON DEVELOPMENTAL NEUROBIOLOGY

Barry J. Hoffer, M.D., Ph.D., Acting Chief

The research efforts of the Section on Developmental Neurobiology have been focused on two broad areas during the past year: 1) The interaction of the adrenergic synapses in the brain with psychoactive drugs and the influence of such adrenergic input upon information processing; 2) the development of synaptic organization during normal ontogeny and after perinatal manipulation. Recently we have also studied 3) mechanisms of cholinergic transmission in the central nervous system and the role of such input in the production of epiliptiform discharge.

THE ROLE OF ADRENERGIC SYNAPSES IN THE BRAIN IN INFORMATION PROCESSING AND THEIR INTERACTION WITH PSYCHOACTIVE DRUGS

Previous work from our group, summarized in last year's annual report, has indicated that cerebellar Purkinje neurons receive an inhibitory adrenergic (NE) input from locus coeruleus and that this inhibition may be mediated by cyclic 3'5'-adenosine monophosphate (cAMP). During the past year, we have studied (Project # Z01 MH 01223) the influence of adrenergic activity on Purkinje cell response to mossy and climbing fiber sensory input. With respect to both excitatory and inhibitory responses, NE clearly increases the magnitude of the response relative to background spontaneous activity and hence increases the "signal to noise" ratio. These findings are especially interesting in view of the postulated role of monoamines in sensory perception and learning.

Another set of experiments in this project concerns the interaction of phenothiazines and their derivatives with the adrenergic input in cerebellum. A wealth of biochemical data has shown that clinically efficacious neuroleptics influence monoamine pathways in the CNS and that, by implication, dysfunction of these pathways may be involved in psychotic behavior. The first problem was to quantitate the responses to microiontophoresis of putative transmitters and the influences of iontophoresis of neuroleptics. Accordingly, a new crystal clock programmable iontophoresis unit and machine language computer programs were developed to quantitate response to iontophoresis of drugs (Freedman, Hoffer and Woodward, Brit. J. Pharmacol., in press). Using this approach, it was shown that clinically efficacious phenothiazines such as fluphenazine and alpha fluphenthixol and chlorpromazine, could selectively antagonize NE and DA responses in cerebellum and caudate respectively (Freedman and Hoffer, J. Neurobiol., in press; Siggins, Hoffer and Ungerstedt, Life Sci., 1974). Clinically inactive phenothiazines showed no adrenolytic activity in cerebellum, either electrophysiologically or biochemically (Skolnick, Daly, Freedman and Hoffer, J. Pharmacol. Exp. Therap., in press). Inhibitory responses to cAMP and GABA are not affected by phenothiazines. Moreover, while parenteral injection of fluphenazine antagonizes the adrenergic inhibition from locus coeruleus, it does not influence GABA mediated basket cell inhibition or climbing and parallel fiber excitation (Freedman and Hoffer, J. Neurobiol., in press). These data strongly support the hypothesis that clinically active phenothiazines specifically influence adrenergic input and are especially intriguing in view of the possible influences of such input on sensory perception noted above.

These experiments represent a continuing effort on the part of the laboratory to characterize the functional role of central adrenergic pathways (Hoffer, Siggins and Bloom, Brain Res., 1971; Hoffer, Siggins, Oliver and Bloom, J. Pharmacol. Exp. Therap., 1973). The current research program is designed to apply what has already been learned in terms of underlying physiological mechanisms to problems in psychopathology and the interpretation of data from clinical psychiatric studies.

THE DEVELOPMENT OF SYNAPSES

A second area studied by our section is the ontogenetic development of chemosensitivity and synaptic organization in the brain. As summarized in previous reports, our initial efforts were directed towards cerebellar "normal developmental milestones" (Woodward, Hoffer, Siggins and Bloom, Brain Res., 1971). More recently, we have produced several perinatal perturbations and examined their effects on subsequent brain development. Using X-irradiation during the neonatal period, it is possible to destroy all cerebellar interneurons without affecting the number of Purkinje cells. The cerebellum remains at less than 1/10 normal adult size and the Purkinje cell bodies and dendritic tree are totally disorganized. Nonetheless, Purkinje cell discharge and chemosensitivity is similar to that of normal cerebellum. Moreover, a synaptic reorganization occurs resulting in both excitatory and inhibitory input to these neurons (Woodward, Hoffer and Altman, J. Neurobiol. 1974). During the coming year, we plan to study cerebellar development in rats suffering from perinatal malnutrition, hypothyroidism, or exposure to antimitotic agents, in order to better define the various environmental influences on brain maturation.

Developmental studies on brain in vivo present many technical problems. However, an extremely useful system for studying neuronal maturation has derived from a technique developed in a collaboration between our section and Dr. Lars Olson of the Karolinska Institute, Stockholm, Sweden. We have successfully transplanted various regions of fetal rat brain into the anterior eye chamber of adult host recipients. The transplants are vascularized from the iris and survive and develop. To date we have extensively studied cerebellum and hippocampus transplants. In both cases, the pieces grow and develop into an adult structure (Hoffer, Seiger, Ljungburg and Olson, Brain Res., 1974; Olson, Freedman, Seiger and Hoffer, in preparation). Histologically, the characteristic laminar neuronal organization is present and electrophysiologically, normal intracortical synapses and chemosensitivity develop.

Significantly, the adrenergic fibers which normally innervate the iris also invade the cerebellar and hippocampal transplants, changing in appearance from "peripheral" to "central" nervous morphology en route, and may establish functional connections with the transplant neurons (Hoffer, Olson, Seiger and Bloom, J. Neurobiol., in press). The adrenergic input to cerebellum in oculo shows the same latency, duration and pharmacological interactions as that described in situ from the locus coeruleus (Hoffer, Siggins, Oliver and Bloom, J. Pharmacol. Exp. Therap., 1973). Thus, the anterior chamber transplants may also provide a way to activate adrenergic input to brain regions by stimulation of the peripheral NE input to the eye. In view of the isolation of the transplant from other central nervous connections, this would provide a degree of selectivity impossible to duplicate in situ. Our future plans include an examination of the interaction between NE input to the transplant, elicited by cervical trunk stimulation, and the administration of psychoactive drugs by superfusion of the anterior eye chamber (see Project Grouping # 1). This would circumvent some of the ambiguities inherent in iontophoresis or parenteral drug administration.

MACROMOLECULAR MECHANISMS IN CHOLINERGIC TRANSMISSION - RELATIONSHIPS TO EPILEPSY

For a number of years, our laboratory has studied the responses to microiontophoresis of NE and the possible role of cAMP as a second messenger in this response. The studies have involved receptor blockade, effects of phosphodiesterase inhibitors, and responses to prostaglandins. Recent studies in the peripheral nervous system have suggested that cAMP and 3'5'-guanosine monophosphate (cGMP) have opposing actions and that cGMP acts as a second messenger for cholinergic transmission. In an effort to extend this dualism to the central nervous system, we have studied iontophoretic responses of pyramidal neurons in the cerebral cortex in situ (Stone, Taylor and Bloom, Science, 1975) and in hippocampal formation transplanted to the anterior eye chamber (Hoffer, Freedman, Olson and Seiger, in preparation). In both cases, the excitatory responses to acetylcholine are mimicked by application of cyclic GMP.

Experimentally and clinically, the hippocampus is characterized by a low threshold for interictal spikes and seizure discharge, and this propensity is retained after transplantation. Indeed, using the EEG, abnormal spikes and seizure discharge which are seen in the transplant after electrical and chemical stimulation are indistinguishable from those found in situ (Freedman, Hoffer, Seiger, Olson and Taylor, in preparation). Our most recent evidence suggests that the seizure thresholds are greatly influenced by cholinergic input to the transplant. Iontophoretic administration of acetylcholine, eserine or cyclic GMP, or activation of the parasympathetic input to the eye, readily triggers interictal spikes and full scale ictal episodes. During the coming year, we plan to study the interaction of cholinergic input with antieplieptic drugs. In view of the extensive cholinergic innervation of the hippocampus and cerebral cortex in situ, two brain areas which are often the sites of epileptic foci clinically, it is hoped that these studies in the transplant will have relevance for understanding the basic neuronal mechanisms in seizure disorders. It may be well to point out that while seizure activity in neurons of the motor cortex produces epileptic convulsions, the behavioral sequelae of seizure activity in (affective) limbic regions of the CNS are more complex. It is not unreasonable to suggest that such abnormal periods of discharge could correlate with hallucinogenic perceptions, amnesia, or other psychopathologic states.

SECTION OF SYNAPTIC PHARMACOLOGY

Forrest F. Weight, M.D., Chief

This summary report for the Section on Synaptic Pharmacology will outline the progress of the Section in achieving its goals and the projected future directions of investigation. One of the major objectives of the Section is the elucidation of the mechanisms of synaptic function and the synaptic basis for drug action in the nervous system. The long range goal of the investigations is an understanding of the mechanisms of neuronal communication and the development of pharmacologic tools to modulate these synaptic mechanisms. Questions investigated on the function of synapses have centered around three major areas: (1) the modulation of transmitter release, (2) the synaptic control of postsynaptic membrane permeability, and (3) the synaptic activation of metabolic systems.

I. HOW IS TRANSMITTER RELEASE MODULATED?

One of the important mechanisms controlling the efficacy of transmission at synapses is the modulation of transmitter release. In order to understand the mechanisms involved, two types of release modulation have been investigated: (1) the role of potassium in the control of transmitter release, and (2) the presynaptic action of other synapses.

Role of Potassium in the Control of Transmitter Resease: At most synapses it is not possible to investigate the mechanisms involved in the modulation of transmitter release because it is not possible to record the electrical activity of presynaptic terminals. However, it is possible to record intracellularly in the presynaptic terminal of the giant synapse of squid. In collaborative studies with Dr. S. D. Erulkar (Univ. of Pennsylvania), this preparation was used to investigate the mechanisms involved in the modulation of transmitter release. The concentration of extracellular potassium (K) ions was found to control the release of transmitter; for example, increasing extracellular K, decreases the release of transmitter. This effect is produced by changes in the amplitude of the presynaptic spike. It is known from the work of Frankenhaeuser and Hodgkin that K accumulates around nerve fibers as the result of repetitive action potentials; this raised the question whether K accumulating as the result of physiological activity would affect transmitter release. It was found that K accumulating around the postsynaptic axon, as the result off repetitive antidromic stimulation, decreases the release of transmitter from the presynaptic terminal. Thus, repetitive activity of the postsynaptic axon has a negative feed-back effect on the release of synaptic transmitter.

Modulation of Transmitter Release by the Presynaptic Action of Other Synapses: Transmitter release may also be modulated by a presynaptic action of other synapses. Dr. A. Padjen has investigated the transmitters involved in presynaptic inhibition in the isolated spinal cord of frog. Using sucrose gap recording, he studied the depolarizing action of neutral amino acids on primary afferent terminals and found that beta-alanine and taurine depolarizations are blocked by strychnine, whereas, GABA depolarizations are antagonized by picrotoxin. The depolarization of primary afferents produced by stimulation of lateral column or dorsal roots is blocked by picrotoxin, but that produced by ventral root stimulation is blocked by strychnine. The results suggest that presynaptic inhibition of primary afferents from dorsal roots or lateral column is mediated by GABA, whereas, the ventral root pathway is mediated by taurine or beta-alanine.

II. HOW DO SYNAPSES CONTROL POSTSYNAPTIC MEMBRANE PERMEABILITY?

In order to study the functional mechanisms of synapses and the interaction with drugs, a multidiciplinary approach has been used to identify and characterize specific synaptic pathways, the synaptic transmitters and the mechanisms of transmitter action. The sympathetic ganglion of the bullfrog was used because of a number of experimental advantages.

Identification and Characterization of Specific Synaptic Pathways, Synaptic Transmitters and Postsynaptic Receptors: In anatomical studies on the ganglion (in collaboration with Dr. H. Weitsen), preganglionic fibers that stain for acetylcholinesterase can be seen to spiral around the axon hillock region of the neuron before termination. In the electron microscope, the preganglionic fibers are found to terminate on the soma of the neuron, a situation well suited for studying the permeability mechanisms of the synapses. With intracellular recording, two types of neurons have been identified in the ganglion, B Cells and C Cells, each type of neuron receives a single monosynaptic connection from different preganglionic fibers. The preganglionic fibers in the sympathetic chain synapse with B Cells, and preganglionic fibers from the eighth nerve synapse with C Cells. Stimulation of these preganglionic fibers elicits a fast excitatory postsynaptic potential (EPSP). Administration of acetylcholine (ACh) by iontophoresis mimics the fast EPSP and these effects are blocked by nicotinic antagonists, indicating the ACh is the transmitter activating nicotinic postsynaptic receptors. In the presence of nicotinic antagonists, repetitive stimulation of the preganglionic fibers produces opposite types of slow synaptic potentials in the different cell types. Stimulation of the sympathetic chain produces a slow EPSP in B Cells and stimulation of the eighth nerve produces a slow inhibitory postsynaptic potential (IPSP) in C Cells. Both the slow EPSP and the slow IPSP are mimicked by the iontophoretic administration of ACh to B and C Cells respectively. The muscarinic antagonist, atropine, blocks these effects indicating that both the slow EPSP and the slow IPSP are mediated by ACh activating muscarinic receptors.

In addition to the cholinergic synapses, there may also be adrenergic synapses in the ganglion. Fluorescence histochemistry reveals that the neurons contain a catecholamine. Chemical analysis (collaboration with Dr. W. Shoemaker) indicates the catecholamine is epinephrine. Using sucrose gap recording, administration of catecholamines (epinephrine, norepinephrine and dopamine) produces a hyperpolarization of the neurons. Pharmacologic analysis indicates that the hyperpolarization is predominantly through the activation of alpha receptors, although beta receptors also contribute.

Fluorescence histochemistry also reveals a small number of small intensely fluorescent (SIF) cells or chromaffin cells in the ganglion. In the electron microscope, these chromaffin cells do not appear to have synaptic connections. Although a synaptic pathway mediated by catecholamines has not been definitely characterized, recent studies suggest that a hyperpolarization produced by antidromic stimulation may be mediated by a catecholamine. This possibility is currently being investigated.

Synaptic Changes of Postsynaptic Membrane Permeability: Synaptic excitation results from an increased permeability of the postsynaptic membrane at most chemically transmitting synapses that have been investigated. This has also been found to be true for the fast EPSP in sympathetic ganglion cells, where the transmitter, ACh, acting on nicotinic receptors, increases the postsynaptic permeability to sodium and potassium ions. Investigation of the slow EPSP revealed that, in contrast to the increased permeability associated with other EPSPs, the slow EPSP is generated by a decrease in membrane permeability. Electrophysiological analysis indicates that the mechanism of generation of the slow EPSP is a decrease in the permeability of the membrane to potassium ions. Because of the *in vitro* nature of the preparation and the fact that the transmitter is known to be ACh, it has been possible to apply ACh iontophoretically and change extracellular potassium ions to obtain additional data supporting this mechanism. Investigation of the slow IPSP has revealed that it is also generated by a decrease in membrane permeability, in contrast to the increased permeability associated with other IPSPs. Electrophysiological analysis of the slow IPSP indicates that the decreased permeability is predominantly to sodium ions. The iontophoretic administration of ACh and changing extracellular sodium has provided supporting data for this mechanism. There are a number of suggestions that similar slow synaptic potentials are involved in other parts of the nervous system; however, because of various technical and experimental limitations, it has not been possible to adequately characterize those mechanisms. Now that the mechanisms have been elucidated in this system, it will be utilized to investigate the molecular basis of these synaptic potentials, and the interactions of drugs.

III. DOES SYNAPTIC ACTIVITY ACTIVATE INTRACELLULAR METABOLIC SYSTEMS?

In an attempt to determine the molecular mechanisms underlying the slow synaptic potentials, the possibility that cyclic nucleotides may act as intracellular second messengers has been investigated. In collaborative experiments with Drs. G. Petzold and P. Greengard (Yale Univ.), it has been found that brief stimulation of the cholinergic preganglionic nerve fibers results in a rapid increase in cyclic guanosine monophosphate (cyclic GMP). When the release of ACh is blocked by high magnesium/low calcium, or when the muscarinic receptors are blocked by atropine, cyclic GMP does not increase. These data indicate that cyclic GMP may be an intracellular mediator of the action of the synaptic transmitter, ACh. In addition, cyclic adenosine monophosphate (cyclic AMP) also increases in the ganglion with synaptic stimulation. The increase in cyclic AMP is also blocked by high magnesium/low calcium, but not by atropine, suggesting that the increase in cyclic AMP may be related to the possible catecholamine synapses that are currently being investigated. These studies suggest that the synaptic transmitter acting on postsynaptic membrane receptors may increase the intracellular content of cyclic nucleotide, which, in turn, results in a change in membrane permeability, thus generating the slow synaptic potential.

FUTURE PROGRAMS

The preceding investigations have identified and characterized specific synaptic pathways, the synaptic transmitters and the mechanisms involved in the synaptic potentials. The following section will briefly outline some of the projected future investigations on the modulation of transmitter release, the molecular mechanisms of synaptic permeability changes and the synaptic basis of drug action.

What are the Mechanisms Modulating Transmitter Release?

One of the future investigations on the modulation of transmitter release will focus on the mechanism of facilitation of transmitter release. It is known that facilitation at the giant synapse of squid is associated with an increase in presynaptic spike height; however, the mechanism of that effect is not known. The investigation will attempt to determine the physical basis of this increase in spike amplitude and whether calcium is involved, since Katz and Miledi have suggested that the storage of calcium in a special "active" form results in facilitation. Also to be investigated, with respect to the modulation of transmitter release, are the effects of ACh, catecholamines and cyclic nucleotides on the release of transmitter from synaptic terminals in the sympathetic ganglion and whether any synaptic pathways mediate such effects.

What is the Molecular Basis of Synaptic Changes in Membrane Permeability?

The primary thrust of future investigations will be an attempt to determine the molecular basis of the synaptic changes in postsynaptic membrane permeability. The studies showing that synaptic stimulation increases cyclic nucleotides in the neurons suggests that the cyclic nucleotides may be involved in the mediation of the slow synaptic potentials. This possibility is being tested by administering the cyclic nucleotides, extracellularly and intracellularly by iontophoresis, to the neurons and determining whether the nucleotidea produce membrane permeability changes that are identical to those involved in the generation of the slow synaptic potentials. In addition, the investigation will include a pharmacologic analysis of phosphodiesterase inhibitors, prostaglandins and other pharmacologic agents that affect the metabolism of cyclic nucleotides.

The molecular mechanism by which an intracellular cyclic nucleotide might produce a change in membrane permeability is not known; however, Greengard has suggested that the nucleotide might activate a protein kinase that results in the phosphorylation of a protein, thus producing a conformational change in a membrane protein. However, direct evidence supporting this hypothesis for the nervous system is, at present, lacking. Since cyclic nucleotides are activated by synaptic stimulation in the ganglion, it is ideally suited for testing this hypothesis. Plans have been made to collaborate with Greengard to determine if synaptic stimulation, ACh, catecholamines or cyclic nucleotides will produce this type of protein phosphorylation.

Another possible molecular mechanism for producing the synaptic changes in membrane permeability is the intracellular translocation of calcium (Ca). Recent studies in other neuronal systems indicate that the level of ionized intracellular Ca may control the membrane permeability for potassium. Since the slow EPSP is due to a decrease in potassium permeability, a change in intracellular Ca might be responsible for its generation. This possibility can be tested by changing Ca concentration extracellularly and intraacellularly, and determining the effect on membrane permeability and the slow EPSP. Such changes in intracellular Ca might also be related to mediation by cyclic nucleotides, since it has been suggested that the cyclic nucleotides may be involved in the intracellular control of Ca.

Recent investigations have revealed that another type of potential is generated in the ganglion cells, a post-tetanic hyperpolarization, that may be due to another molecular mechanism - the electrogenic sodium (Na) pump. In future investigations, we plan to characterize this phenomenon and determine whether the electrogenic Na pump is involved. This will be particularly important in relation to the mechanism of generation of the slow IPSP and the catecholamine hyperpolarizations.

Does Synaptic Activity Result in Long Term Molecular Changes?

Recent studies in other laboratories have suggested that increased activity in preganglionic afferents to sympathetic ganglia results in an increase in the enzyme tyrosine hydroxylase. The experiments are somewhat indirect and a number of variables are not controlled. The *in vitro* sympathetic ganglion is ideally suited for a more detailed investigation of such phenomena, because the preganglionic fibers can be selectively stimulated and the ionic and chemical environment can be precisely controlled. If appropriate collaborative arrangements for the biochemistry can be worked out, we will investigate whether prolonged synaptic stimulation will result in the trans-synaptic induction of enzymes or other molecules. This investigation may lead to a greater understanding of long term molecular changes in neurons resulting from synaptic activity.

What is the Synaptic Basis of Psychotropic Drug Action?

In view of the preceding information on selective synaptic pathways with known synaptic mechanisms, we are in an ideal position for evaluating the synaptic basis of drug actions. Of particular relevance are the actions of various psychotropic agents used in the clinical treatment of psychoses and depression. For example, lithium (Li), used in the treatment of manic-depressive psychosis, has been found to selectively block the slow IPSP, but does not affect the fast or slow EPSP. We are currently investigating the mechanism of this selective lithium action. We also plan to test other psychotherapeutic drugs such as phenothiazines and tri-cyclic antidepressants, in an attempt to determine whether they may act by mechanisms such as: modulating transmitter release, blocking the action of a transmitter on postsynaptic receptors, altering the membrane permeability changes produced by receptor activation or affecting intracellular molecular pathways.

CONCLUSIONS

The preceding summary indicates that fundamental neurobiological investigations have revealed defined synaptic pathways that have permitted elucidation of the mechanisms of synaptic transmission. Such test systems will be used in future investigations to attempt to determine the molecular mechanisms involved in the synaptic control of membrane permeability and the synaptic basis for drug actions in the nervous system.

Laboratory of Clinical Psychopharmacology

Richard Jed Wyatt, M.D., Acting Chief

The basic goal of the Laboratory has been to identify, understand, and solve significant mental health problems. Specifically, we are interested in schizophrenia, drugs of abuse, sleep research and aging. We have attempted to bring the most sophisticated and appropriate methods of biological psychiatry to bear on these problems. In this effort, we have been fortunate in developing excellent collaborative relationships with other basic and clinical laboratories in the Intramural Program, in particular, the Laboratory of Preclinical Pharmacology, the Laboratory of Clinical Science, and the Adult Psychiatry Branch.

During FY 1974, Dr. J. Christian Gillin was Temporary Acting Chief of the Laboratory while I was at Stanford University. He continued the reorganization which I had begun three years ago and which is now (FY 1975) almost complete. This organization was designed to integrate as much of the research in the Laboratory as possible, making maximal use of all personnel and other resources. This means that patients are studied simultaneously by a number of techniques and that the staff members have a good familiarity with each of these techniques.

Studies of Schizophrenia

Studies with chronic schizophrenic patients have continued to be the most important aspect of the Laboratory's efforts. Our major finding (together with Dr. Dennis Murphy) is that platelet monoamine oxidase activity is low in chronic schizophrenic patients and that this deficit seems to be genetically determined. Numerous studies have attempted to show other causes of this deficit but have so far failed. The major effort of the Laboratory is to determine the meaning of this finding and to discover if it is related to the cause of the illness. Toward this end, Dr. Farouk Karoum has developed gas chromatographic-mass spectrometric assays for many of the monoamines and their metabolites in blood and spinal fluid. These assay procedures are currently being applied to samples from many patients and normal controls.

Further efforts are being made to understand the complex relationships that seem to exist between the indole and catecholamines in schizophrenic patients. Our initial finding that some patients have moderate clinical improvement with 5HTP administration (the serotonin precursor) has now also been demonstrated in the presence of neuroleptic drugs. This improvement however, is complicated by a superimposed toxic effect. These effects occurring in 6 of 8 patients include confusion, transient activation of psychosis and extrapyramidal signs. We are trying to learn how to eliminate these toxic effects.

A number of substances have been proposed as possible endogenous schizotoxens. We have begun testing these substances according to the following criteria:

1. The substance must be capable of mimicking clinical aspects of schizophrenia;
2. The substance must be found in man;
3. The precursor of the substance must be found in man (see #4);
4. The substance should be synthesized in man (it remains a possibility that the substance could be of dietary origin;
5. The substance must be differentially synthesized or metabolized in schizophrenics;
6. Tolerance to the substance should not develop slow tolerance to the agent might be acceptable for acute schizophrenia but not for the chronic illness. (This assumes the agent does not produce irreversible damage.);
7. Neuroleptic drugs must be capable of antagonizing the synthesis, increasing the metabolism, or antagonizing the effect of the agent.

Our and other laboratories have demonstrated that DMT meets at least 5 of the 7 criteria. Number 7 has not been tested. Number 5 has been confirmed in some laboratories, but not in ours.

The bovine pineal extract previously given intramuscularly with success in some patients is now being tested orally. From the crude substance, inhibitory activity against a rabbit lung, DMT-forming enzyme has been found. Fractionation of the extract for its inhibitory activity is being attempted.

An enzyme capable of forming tyrptoline and related compounds has been identified in human platelet and brain. This enzyme uses the carbon donor 5-MTHF as a cofactor.

Dr. Reynolds has continued his empirical construction of predictive models of schizophrenic behavior. Collecting the incidence of simple behaviors such as pacing in chronic schizophrenic patients over weeks and months, he is able to predict future occurrences of the behavior. Until now, for each patient a new set of equations has had to be derived. During the last year, mathematical equations have been used which may be able to predict behavior across patients. It is hoped that this technique will be able to meaningfully compare time series studies of one patient with another.

Drugs of Abuse

These studies have centered around question of why people use marijuana and other drugs of abuse. Marijuana, like other drugs of abuse, shows state dependent effects, particularly with regard to learning.

In the case of state dependent learning, what a subject learns while "straight" may not be remembered after he has smoked marijuana and vice versa. On the other hand, what a subject learns after smoking marijuana is better remembered after smoking marijuana. This change in state produced by marijuana is important to understand further. Toward this end, we have learned that the "high" produced by marijuana is different than that produced by opiates. The latter produces euphoria not seen in marijuana. It seems that the change in state surrounding marijuana use is a sought for opportunity to get out of the "straight" situation.

Sleep Studies

We are currently in charge of running the human sleep laboratory at the Clinical Center in a joint effort with the clinical units there. For simplicity, our total sleep effort has been consolidated into one report which will appear under the Adult Psychiatry Branch.

Studies of Aging

Little substantive progress on our aging project has been made during the last year. The book, Dementia in the Presenium, has been published and been well reviewed. A second book on care of the elderly is well underway. A few patients with low pressure hydrocephalus have been studied to determine if we can predict who will respond to surgery, but without any reportable results as of yet.

LABORATORY OF PRECLINICAL PHARMACOLOGY

E. Costa, M.D., Chief

There has been no slack in the number of original contributions made by the laboratory during Fiscal Year 1975. The major ones were: 1) ^{18}O was used to measure the in vivo turnover rate of dopamine (DA) in brain nuclei isolated stereomicroscopically which contain cell bodies (A_9 or A_{10}) or nerve terminals of DA neurons; 2) the physiological role of various molecular forms of monoamineoxidase (MAO) has been characterized; 3) an endogenous progein activator of phosphodiesterase regulates the K_m of cyclic nucleotide phosphodiesterase (PDE) and participates in the control of the steady state content of 3',5'-cyclic adenosine monophosphate (cAMP); 4) the activation of protein kinase by cyclic nucleotides mediates the trans-synaptic induction of TH in adrenal medulla; 5) the synthesis rate of tyrosine hydroxylase (TH) increases during the trans-synaptic induction of TH in adrenal medulla and the number of hydroxyindole-O-methyltransferase (HIOMT) molecules accumulate during trans-synaptic regulation of this enzyme in pineal; 6) striatal acetylcholine (ACh) turnover rate is increased by antipsychotics that cause extrapyramidal side effects; 7) the in vivo increase of cAMP content and acetylcholine (ACh) turnover rate in specific brain nuclei was used as an index to study drug mechanisms at synaptic level; 8) new high pressure liquid chromatography (HPLC) and mass spectrometric (MS) methods developed in our laboratory have revealed that halogenated amphetamine analogues exert a selective neurotoxicity on certain serotonergic brain nuclei; 9) an activation of GABA receptors participates in the presynaptic depolarization, anticonvulsant and antitremorigenic action of benzodiazepines; 10) a N-acetyltransferase which can be inhibited by beta carbolines was described in brain tissues.

During Fiscal Year 1975, the laboratory was organized into four sections. The activities of these sections were coordinated by a unit concerned with the neurobiological application of MS (mass spectrometry) and HPLC (high pressure liquid chromatography). The section denominations are: 1) Biochemical Pharmacology; 2) Neuroendocrinology; 3) Molecular Neurobiology; and 4) Molecular Pharmacodynamics.

Section on Biochemical Pharmacology (Chief: N. H. Neff, Ph.D.)

The major emphasis in the activities of this section was an evaluation of the function of the various molecular forms of MAO that participate in synaptic transmission regulation. In the past, this section had investigated the biochemical and pharmacological characteristics of various forms of brain MAO.

These studies suggested that certain forms of MAO preferentially participate in the modulation of selective brain functions. Hence, by using specific inhibitors which block certain forms of MAO preferentially, relationships could be established between the regulation of specific brain functions and the catalytic activity of certain molecular forms of MAO (Type A and B enzymes).

Dr. Fuentes has provided evidence in support of the hypothesis that only the inhibitors of Type A MAO can prevent the depression of motor activity induced in rats by a treatment with reserpine. Assuming that the depression of motor activity elicited by reserpine is an acceptable experimental model to evaluate drug actions in human depression, one might infer that a blockade of type A enzyme is potentially more useful to relieve the signs of depression in man than the blockade of type B MAO or the use of other MAO inhibitors which indiscriminately block both enzymes. Most of the MAO inhibitor drugs that are currently used in the clinic belong to this class.

Tranylcypromine is a MAO inhibitor with antidepressant properties; there are two stereoisomers of this compound and the drug given to patients is a mixture of the (-) and (+) isomers. Some reports suggest that the antidepressant activity of tranylcypromine might reside with the (-) isomer because it is apparently more potent than the (+) isomer in inhibiting the amine reuptake by nerve endings. Dr. Fuentes and Dr. Oleshansky found that in these isomers of tranylcypromine the ability to relieve the depression elicited by reserpine correlates with the blockade of MAO better than with the blockade of amine reuptake. These studies may provide a new framework of reference for developing and evaluating antidepressant drugs.

The activity of PDE increases in isolated cell systems when the concentrations of cAMP are increased following the stimulation of specific receptors. This finding suggests that cAMP might regulate the activity of the only enzyme that catalyzes its own destruction. Since the injection of beta-adrenergic agonists increases the cAMP content in the pineal gland of rats, Dr. Oleshansky studied whether l-isoproterenol treatment resulted in an increase of PDE in pineal homogenates. He found an increase in PDE activity which could be prevented by pretreatment with beta-adrenergic receptor antagonists. These studies imply that when cAMP content increases following beta receptor activation, the high pineal content of this nucleotide triggers some mechanisms which influence its own destruction. This type of autoregulation may explain the tolerance that develops to repeated administration of some adrenergic receptor agonists.

The activity of HIOMT in pineal of rats shows a diurnal rhythm which is regulated, in part, by sympathetic nerve activity. During darkness, the activity of this enzyme is higher than during light. By using immunochemical titration with antiserum to this enzyme, Dr. Yang demonstrated that an increased number of enzyme molecules rather than a change in enzyme kinetics correlates with the increase in enzyme activity during continuous darkness. Apparently, the enzyme protein level is controlled in part by sympathetic nerve activity. Dr. Yang found that the N-acetyltransferase of brain acetylates a wide variety of naturally occurring biogenic amines as well as amphetamine and that the enzyme was inhibited by beta-carboline derivatives. It is important to note that Dr. R. Wyatt (Lab. of Clinical Psychopharmacology) has shown that such beta-carboline derivatives can be formed as a result of serotonin metabolism; thus a possible mechanism that may be operative in mental illness can be suggested. Liver and brain N-acetyltransferase have similar characteristics while pineal N-acetyltransferase have different substrate and inhibitor requirements. The presence of this enzyme in brain may explain why the brain melatonin content remains unaltered following pinealectomy. Moreover, this enzyme may provide an alternative pathway for the metabolism of a yet undisclosed number of endogenous amines.

Tardive dyskinesia is induced in man by treatment with neuroleptic drugs. The disease can be alleviated by treatment with 2-dimethylaminoethanol (DMAE); however, the mechanism of action of the drug is unknown. Dr.Garrison found that DMAE is a substrate for choline acetyltransferase. These results suggest that DMAE acetate might be formed in cholinergic neurons and functions as a false neurotransmitter, thus, reducing the physiological effectiveness of the cholinergic system. This hypothesis is especially inviting since other members of this laboratory have demonstrated that acetylcholine synthesis is accelerated after treatment with neuroleptic drugs.

Section on Neuroendocrinology (Chief: A. Guidotti, M.D.)

Dr. Zivkovic and Dr. Biggio have developed a method to measure the protein kinase activity (cAMP dependent and independent) in stereomicroscopically isolated hypothalamic nuclei. The ratio of the two activities $(\frac{-\text{cAMP}}{+\text{cAMP}})$ normally is 0.2 in several hypothalamic nuclei studied. In certain nuclei the ratio increases to about 0.6 during the stimulation of pituitary function elicited by reserpine or cold exposure. It appears possible to use this ratio as an index to locate the structures involved in pituitary regulation and to study the biochemistry of neuroendocrine regulation at synaptic level.

Dr. Kurosawa demonstrated that in adrenal medulla homogenates an increase of cAMP independent protein kinase activity precedes the induction of TH elicited by cold exposure, carbachol and reserpine injections. Using various doses of either carbachol or reserpine the induction of TH and the activation of protein kinase measured at 24 and 1.5 hours, respectively show an identical dose response relationship. In all cases studied, the activation of protein kinase is preceded by an increase of cAMP content, its onset is coupled with the stimulus but this increase is independent from stimulus duration and never lasts longer than 90 minutes. The extent of the stimulus coupled increase of cAMP content is dose related but the threshold of carbachol to elicit this increase is lower than that required to either activate protein kinase or to induce TH. The time duration of the cAMP increase appears to participate in the protein kinase activation and the TH induction. The activation of protein kinase persists for about 4 hrs and it is followed by a translocation of the activated enzyme to a particulate fraction which includes the nuclei. Recently we have obtained translocation of a histone kinase into the purified nuclear fraction at 4 hrs after the application of stimuli that induce tyrosine hydroxylase trans-synaptically. We are now proceeding in the characterization of the endogenous nuclear substrate for this protein kinase to uncover the molecular basis for the increase in DNA transcription associated with the trans-synaptic induction of TH. In collaboration with Dr. I. Hanbauer (NHLI), we are studying the mechanism of the trans-synaptic induction of TH in rat superior cervical ganglia. Here two major mechanisms appear of importance: the activation of a glucocorticoid receptor which appears to have a permissive action on the induction and the persistent stimulation of a nicotinic receptor which causes a decrease of the cGMP content of ganglia. This nicotinic receptor activation which leads to an induction of TH is counteracted by the release of dopamine (DA) from SIF cells. The release of DA is triggered by the activation of muscarinic receptors. In addition, the induction of ganglionic TH can be mediated by an activation of beta adrenergic receptors which occurs when the blood borne epinephrine reaching the ganglia increases. This activation of beta adrenergic receptors causes an increase of cAMP content, thus, unlike adrenal medulla, the induction of TH in ganglia can be preceded by either an increase of cAMP or a decrease of cGMP which are coupled with the stimulation of beta adrenergic and nicotinic receptors, respectively.

In collaboration with the Laboratory of Clinical Psychopharmacology, members of this section have studied the adenylate cyclase activity in brain of schizophrenics. The enzyme can be measured at several hours after death and the enzyme can still be activated by dopamine (DA). The adenylyl cyclase in striatum of the patients who died while taking phenothiazine antipsychotics is resistent to the activation by DA.

Dr. Zivkovic has continued the study of the short term regulation of TH in striatum and N. accumbens of rats receiving various antipsychotics. He has established that drugs possessing a high liability to cause extrapyramidal side effects, preferentially increase the affinity of striatal TH for the pterine cofactor. Drugs that fail to cause extrapyramidal side effects preferentially increase the affinity of N. accumbens TH for the pterine cofactor. By appropriate experimental design, Dr. Zivkovic demonstrated that cholinergic synapses participate in the multineuronal loop that regulates the activity of dopaminergic neurons of the nigro striatal pathway. We are now studying whether or not a cholinergic synapse participates in the feedback regulation of mesolimbic dopaminergic neurons.

It has been proposed that in dopaminergic nerve terminals there are specific receptors to DA (dopaminergic autoreceptors) which regulate the function of dopaminergic terminals. Dr. Zivkovic's experiments have shown that these DA autoreceptors play no role in the regulation of the kinetic state of striatal TH. This regulation appears to depend on the firing rate of the dopaminergic neurons. The GABA system is involved in the feedback regulation of dopaminergic activity in striatum and N. accumbens. GABA-ergic mechanisms may represent an important inhibitory mechanism participating in the regulation of dopaminergic neurons. Perhaps, agonists of GABA receptors could normalize the enhanced activity of DA neurons which is currently believed to be associated with the appearance of certain symptoms which are typical of schizophrenia.

Dr. Mao had shown that during cold exposure that 3',5'-cyclic guanosine monophosphate (cGMP) content of cerebellum is increased. This increase can be prevented by a facilitation of GABA release from neurons. Moreover, Dr. Mao had shown that a similar increase of cerebellar cGMP could be obtained by injecting harmaline which selectively activates the olivo-cerebellar pathway (climbing fibers). Also the injection of blockers of glutamic acid decarboxylase (GAD) increases cerebellar cGMP content. Dr. Biggio has shown that the injection of 3-acetylpyridine which causes a selective degeneration of climbing fibers, prevents the increase of cerebellar cGMP elicited by harmaline or cold exposure, but not that elicited by the injection of GAD inhibitors. Our working hypothesis is that the activation of climbing fibers results in the trans-synaptic stimulation of a guanylcyclase presumably located in the membranes of the Purkinje cell dendrites. This possibility is supported by studies with mutant mice performed by Mao and Landis (Lab. Neuropharmacology). These mutant mice possess a selective reduction in the number of Purkinje cells (approximately 10% of normals) and the cGMP in these cerebella is reduced by 60% and fails to increase following climbing fiber activation. The cAMP content of these mutant mice cerebella is normal. When climbing fibers are destroyed by 3-acetylpyridine a decrease of GABA content elicited by GAD blockers can still elicit an inincrease of cerebellar cGMP. Dr. Biggio and Dr. Naik are currently investigating whether parallel fiber activation also leads to cerebellar guanylcyclase activation. Dr. Mao had shown that the intraventricular injection of GABA but not that of glycine reduced the cerebellar content of cGMP; Dr. Naik has shown that this effect is mimicked by the parental administration of GABA receptor agonists. In contrast, various types of manipulations of monoaminergic or cholinergic neuronal systems failed to change the cerebellar cGMP content or to reduce the increase of this cyclic nucleotide elicited by harmaline or cold exposure. Harmaline elicits tremor which persists unabated in rats receiving cholinergic receptor blockers but it is inhibited by GABA receptor agonists, by the injection of diazepam or other anxiolytic benzodiazepines and by the destruction of climbing fibers with 3-acetylpyridine. Dr. Mao and Dr. Biggio have compared the potency of various benzodiazepines to decrease cerebellar cGMP, they found that anxiolytic benzodiazepines are at least 100-fold more potent than phenobarbital in decreasing the content of this nucleotide in cerebellum. Dr. Biggio is studying the action of various drugs on the cGMP content of cerebellar molecular layer and deep cerebellar nuclei. Harmaline preferentially increases the cGMP content of the molecular layer. In contrast, GAD blockers indiscriminately increase the cGMP content of both cerebellar structures. Dr. Biggio is currently investigating with some detail the mode of action of benzodiazepines and found that these drugs decrease the cGMP content in the molecular layer and in the deep nuclei of cerebellum. Since the action of benzodiazepines does not require the presence of climbing fibers we have postulated that benzodiazepines act by releasing GABA. To provide additional evidence in support of this view, Dr. Mao has studied with some detail the spectrum of the anticonvulsant action of benzodiazepines. He has used GAD inhibitors, two GABA receptor blockers (picrotoxin and pentylenetetrazol) and a glycine receptor antagonist (strychnine): all the benzodiazepines tested were ten-fold more active in preventing the convulsions elicited by GAD inhibitors than in antagonizing the action of picrotoxin and pentylenetetrazol. The benzodiazepines potency against strychnine convulsions was at least 60 times weaker than that against convulsions elicited by GAD inhibitors. The hypothesis that a facilitation of GABA transmission participates in the action of benzodiazepines was therefore supported. On the basis of this evidence we have suggested that also the anxiolytic action of benzodiazepines may be related to a release of GABA. Direct experiments were carried out by Dr. L. Stein (Wyeth Laboratories) which provided evidence that the increase in punished behavior elicited by benzodiazepines (oxazepam) can be antagonized by pictrotoxin, a GABA receptor antagonist but not by strychnine, a glycine receptor agonist. These experiments establish the involvement of GABA mechanisms in the relief of anxiety and indicate that benzodiazepines are important tools for the exploration of GABA's role in psychopathology.

Section on Molecular Pharmacodynamics (Acting Chief: D. L. Cheney, Ph.D.)

Dr. A. Suria has investigated the mode of action of benzodiazepines using as a model the sympathetic chain of bullfrog and as a recording procedure the sucrose gap method. It was reported that GABA (10 μM) applied to this preparation causes a depolarization of the cholinergic neuron terminals. Diazepam mimicked this action of endogenous GABA. Sumilarly to the action of GABA, the depolarization elicited by this benzodiazepine can be blocked by picrotoxin and bicuculline. The depolarization elicited by diazepam, unlike that of GABA, is blocked by GAD inhibitors. This finding suggested to Dr. Suria that diazepam may increase the extracellular concentrations of GABA thus facilitating its interaction with cholinergic nerve terminals impinging on the ganglion. It is known that the sympathetic ganglia of bullfrog do not contain GABA neurons, but GABA is taken up by a Ca++ dependent high affinity uptake into glial cells. The stimulation of the ganglion and the addition of depolarizing concentrations of K+ causes a release of ^{3}H GABA taken up by this tissue. Dr. Suria then tested whether benzodiazepines could block the uptake of radioactive GABA; the results of this experiment were negative but he could show that benzodiazepines can release GABA. This experiment indicated that the presynaptic depolarization of nerve terminals elicited by benzodiazepines may be mediated via a release of GABA from glial stores. Dr. Bertilsson is measuring the GABA and glutamic acid content of the sympathetic chain by MS.

Mr. Richard Lehne has studied with some detail the action of benzodiazepines on the catabolism of cAMP and cGMP by an activator free PDE in the presence and in the absence of the protein activator of PDE extracted from frog ganglia. Diazepam and other benzodiazepines appear to inhibit PDE in the native or activated form by competing with the substrate. The K_i values of benzodiazepines are generally lower for the activated form of the enzyme. Since Dr. Suria has shown that cGMP mimics the depolarizing action of GABA in presynaptic terminals and that cGMP and diazepam can release GABA from ganglia it is tempting to postulate that the release of GABA stores by diazepam be mediated via an inhibition of the metabolism of cGMP.

Dr. Racagni and Dr. LeFevre have developed a technique to measure the acetylcholine (ACh) content of stereomicroscopically dissected nuclei of rat brain. Thus, using MS, it has become possible for the first time to describe in detail the distribution of cholinergic neurons in various brain nuclei. By combining measurements of ACh by MS with the assay of choline acetyltransferase (CAT) it appears possible to formulate some prediction as to the prevalent structures that are present in a given nucleus. In the nucleus motorius vagi which contains abundant cholinergic cell bodies the ratio $\frac{CAT}{ACh}$ has very small values, these values are elevated by two or three orders of magnitude when samples such as corpus callosum containing axons exclusively are analyzed.

The application of MS to the study of cholinergic mechanisms has been extended to measure turnover rate of ACh in discrete brain nuclei. Dr. Zsilla and Dr. Racagni have measured the turnover rate of acetylcholine in N. accumbens and N. interpeducularis; thus establishing the first method to measure at synaptic level the effect of drugs on the dynamic equilibrium that regulates cholinergic mechanisms. Presently the action of opiates on the ACh synthesis at synapses is being investigated. Dr. Racagni has pursued the study of the mode of action of various antipsychotics on the cholinergic function of striatum. Clozapine, which fails to cause tardive dyskenesia or extrapyramidal side effects, differs from the antipsychotics which possess a high extrapyramidal liability because it fails to increase the turnover rate of striatal ACh. In addition, Dr. Racagni has shown that apomorphine and oxotremorine reduce striatal ACh turnover. Both actions can be blocked by clozapine in doses that fail to change striatal ACh turnover. Clozapine can block dopaminergic receptors by a mechanism similar to that of haloperidol and chlorpromazine but, in addition, it can block cholinergic postsynaptic receptors in vivo.

Dr. Racagni has presented the first in vivo evidence that by virtue of its anticholinergic activity clozapine is endowed with a minimal extrapyramidal liability. This information and the methods developed in this section during FY 1975 have introduced a novel dimension in predicting whether new antipsychotics with a therapeutic activity analogous to chlorpromazine may have a limited capacity to produce extrapyramidal side effects.

Section on Molecular Neurobiology (Acting Chief: E. Costa, M.D.)

The regulation of the increase of cAMP and CGMP content elicited trans-synaptically in postsynaptic cells depends on the equilibrium between the catalytic activity of two enzymes: the nucleotide cyclase located on the postsynaptic membrane and the cyclic nucleotide PDE. The kinetic state of both enzymes can be influenced: that of the cyclase by the transmitter, that of the PDE by a protein activator (PA). Dr. Uzunov has developed an original technique for the measurement of the thermostable PA (M.W. 20.000) which increases the catalytic activity of a PA deficient PDE. This enzyme is isolated from rat brain by polyacrylamide gel electrophoresis. Kinetically, this purified PDE is characterized by a high K_m for cAMP and a low K_m for cGMP. Dr. Uzunov and Dr. Moja (Lab. Clinical Psychopharmacology) have measured the PA in stereomicroscopically isolated brain nuclei. It appears that three antidepressants: desmethylimipramine, iprindol and amytryptiline, decrease the activity of the PA present in brain nuclei containing noradrenergic nerve terminals while chlorpromazine increases it. Dr. Uzunov and Dr. Moja are currently using as a working hypothesis that the inhibition of norepinephrine (NE) reuptake is not the only mechanism that participates in the therapeutic action of antidepressants. In fact, iprindol which in the clinic has an antidepressant efficacy similar to that of desmethylimpramine is devoid of inhibitory capacity on the reuptake of NE. Drs. Uzunov and Revuelta have shown that in the adrenal medulla the activity of the PA increases when the cAMP content of chromaffin cells is elevated following the stimulation of nicotinic receptors. The elevation of the PA activity follows the increase of cAMP content after a time delay. Drs. Uzunov and Gnegy have purified the PA of PDE to chromatographic homogeneity and produced an antiserum to the PA in goats. With this tool, it will be possible to study in detail the location and the regulation of the PA.

Drs. Uzunov, Gnegy and Mr. Lehne have studied the kinetic properties of PDE in the presence and in the absence of the activator, using cAMP and cGMP as a substrate. This protein modifies the kinetic properties of the molecular forms of PDE which have a high K_m for cAMP. The activator lowers this K_m by about 6 to 8-fold, optimizing the catalytic properties of the enzyme in the range of intracellular concentrations of cAMP. We have not yet understood how the increase of cAMP brings about an increment in the activity of the PA. However, we are learning some interesting biochemical properties of the PA. This protein is not a substrate for protein kinase but it inhibits histone phosphorylation by a protein kinase purified from beef heart. This purified protein kinase contains PDE activity. This PDE has a high K_m for cAMP and the enzyme can be phosphorylated in the presence of cAMP and ATP. The phosphorylated enzyme has a low catalytic activity. The PA of PDE stimulates protein phosphatase and in the presence of protein phosphatase and cAMP the K_m of the PDE for cAMP is decreased. We are presently considering that the regulation of cAMP catabolism depends on a supramolecular entity which includes: 1) the PA; 2) a PDE; 3) a protein kinase and 4) a protein phosphatase. When the intracellular cAMP content increases the number of PA molecules may increase; thus, the phosphorylation of PDE is inhibited and the action of protein phosphatases is facilitated. The dephosphorylated PDE has a low K_m for cAMP and rapidly reduces cAMP levels. Dephosphorylation of PDE is reduced when the cAMP content is reduced while the enzyme phosphorylation which increases the K_m of PDE occurs when the PA levels are reduced. As long as PA levels are elevated, phosphorylation of phosphodiesterase is impaired. The presence of low K_m phosphodiesterase may participate in making the receptor cells resistent to incoming stimuli. If our model is substantiated by experiments still in progress, it can offer an original interpretation of the regulation of second messenger responses including the time constant of the second messenger responses following continuous stimulation; habituation in synaptic responses may also be explained.

Dr. D. Chuang has obtained the first measurement of the rate of synthesis of TH in adrenal medulla. immunochemical affinity chromatography, he has found that TH has a t 1/2 of 68 hours. During the trans-synaptic induction of TH the synthesis rate of the enzyme increases whereas the degradation rate of the enzyme fails to change. Dr. Chuang has measured the rate of uridine incorporation into ribonucleic acids of adrenal medulla and has found that this synthesis begins to increase at six hours after the application of the inducing stimulus, the peak increase of RNA synthesis occurs at 8 hrs. He has also produced preliminary evidence that RNA polymerase II is increased. These studies are still in progress. The TH synthesis is increased after about 10 hours from the stimulus application and returns to normal after about 48 hours. Due to the slow turnover time of the enzyme the enzyme activity returns to normal level after about 8 days following the cessation of the increase in the enzyme synthesis rate.

Unit on Neurobiological Applications of MS and HPLC

Drs. Koslow and LeFevre have developed MS methods and have executed the measurements of DA turnover rate in brain nuclei using inhalation of $^{18}O_2$ to label the DA at the rate limiting step. They found that the turnover rate of DA is much faster in brain nuclei containing cell bodies than in those which contain nerve terminals. The percent of variant O_2 present in DA is greater than that present in homovanillic acid and 3,4-dihydroxyphenylacetic acid. This finding argues against the hypothesis that DA in dopaminergic neurons is compartmentalized into a metabolic and storage pool and that only the metabolic pool of DA participates in neuronal activity. With this technique, it can now be initiated the study of the drug actions on the metabolic compartmentation of DA at synaptic level. Moreover they have terminated the preparatory work to initiate the measurement of DA turnover in man using ^{18}O. This collaborative project with the Laboratory of Clinical Psychopharmacology will be carried out in FY 1976.

Dr. Bertilsson had conducted experiments to ascertain whether halogenated derivatives of amphetamine cause selective and irreversible lesions in brain DA neurons. His findings support the hypothesis that p-chloroamphetamine causes an irreversible damage of serotonergic neurons. He found that the serotonin content in brain nuclei which contain terminal axons is decreased for longer than 1 month after a single high dose of the drug. The serotonin content of the raphe nuclei is normal except in the area B9 where the transmitter is decreased for longer than 1 month. A single high dose of fenfluramine, an anorectic agent, used in the clinic, causes a long lasting depletion of serotonin but the damage acquires a permanent character neither in the cell body nor in the nerve terminal.

Dr. Neckers performed parallel studies by measuring the tryptophan hydroxylase activity in the same areas and in the identical experimental conditions studied by Dr. Bertilsson. These studies were made possible by the enzymatic methods developed by Dr. Meek using HPLC to detect the product formed, 5-hydroxytryptophan. Dr. Necker's results were in good agreement with those of Dr. Bertilsson, thus confirming the possibility that a retrograde lesion preferentially extends to the cell bodies of the B9 area, following a single injection of p-Cl-amphetamine. The mechanisms of this lesion and the reasons for this selectivity are currently being investigated. Dr. Meek has used the powerful HPLC enzymatic assay for tyrptophan hydroxylase to evaluate in some detail the areas of innervation of various serotonergic neuronal groups. The combination of specific electrical lesions with this new enzyme assay may reveal details in the distribution of serotonergic neurons that may surpass in precision the histofluorescence technique for the detection of serotonin. Dr. Meek has developed a HPLC assay for the detection of taurine. This method is specific, sensitive and rapid. The practical value of such a method derives from the possible involvement of a CNS taurine deficiency in at least one recently genetic determined form of depression.

PROJECTIONS FOR 1976

The laboratory has been organized as shown in the following scheme.

Office of the Chief:
Joan A. Danzig
Mary Adams
Bernadette Williams

Unit on Neurobilogical Applications of MS and HPLC

J. L. Meek, Ph.D.
L. Neckers, Ph. D. (NIMH Guest Worker)
S. Lofstrandh
H. LeFevre, Ph. D.
L. Bertilsson, Ph.D.

Section on Biochemical Pharmacology (Chief: N. H. Neff, Ph.D.)

N. H. Neff, Ph.D.
M. Oleshansky, M. D.
C. Garrison, Ph.D.
J. Rubenstein
L. Quenser, Ph.D. (NIMH Guest Worker)

Section on Neuroendocrinology (Chief, A. Guidotti, M.D.)

A. Guidotti, M. D.
A. Kurosawa, Ph.D.
A. Naik, Ph.D.
G. Biggio, Ph. D.
K. Gale, Ph. D. (PRAT)
M. Allen

Section on Molecular Pharmacodynamics (Acting Chief: D. L. Cheney, Ph. D.)

D. L. Cheney
G. Racagni, Ph.D.
A. Suria, Ph.D.
G. Zsilla, Ph. D.
R. Shirasawa
R. Lehne

H. Yang, Ph. D.
D. Chuang, Ph. D.
A. Revuelta, D.D.S.
M. Gnegy, Ph. D.
P. Uzunov, Ph.D., M. D.
J. S. Hong, Ph.D. (NIMH Guest Worker)
R. A. Hollenbeck, Ph.D. (NIMH Guest Worker)

However, to keep the operation at this level of productivity and efficiency, I must request some changes in the staffing. Until now the resources available to staff the laboratory are 16 permanent positions and 11 other than permanent positions. The 11 other than permanent positions were used to hire visiting associates and visiting scientists. With the enforcement of the new regulations concerning 1 year appointments, we lose the possibility of using the 11 other than permanent positions for this purpose. To optimize the resources it would seem appropriate that four of the other than permanent positions be substituted by four permanent positions. Thus, the resources available would become 20 permanent positions and 7 other than permanent positions. During the last three years, it has become possible to finance the activities of the laboratory by virtue of special support from the drug abuse programs of ADAMHA. In view of the possibility that such additional support will not materialize, it would be helpful if the annual support of the laboratory could be increased from $180,000 to $220,000. It is needless to emphasize that we are operating 3 mass spectrometers without special additional support. This increase could, in part, be defrayed if, when we are allowed to submit requests for research support to NIDA, moneys for support of our mass spectrometry units is permitted.

Annual Report of the Section on Technical Development

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

July 1, 1974 - June 30, 1975

Theodore R. Colburn, Ph.D., Chief

The Section on Technical Development is a group of engineers, computer specialists, and technicians which provides technical services to the Intramural Research Programs of NIMH and NINCDS. The major functions of the Section are:

(1) Instrumentation research and development. Design and development of instruments and instrumentation systems which represent advances in the state-of-the-art. Most of the research within the Section falls in this category, and is generally done in collaboration with investigators in the laboratories of NIMH and NINCDS.

(2) Production of custom instrumentation. Design and fabrication of electronic, mechanical, and optical equipment to suit the particular needs of the requesting investigator. These instruments, while often quite complex, utilize rather than advance the current state-of-the-art design techniques and components.

(3) Computer services. The Section assists the investigators in data collection, reduction, and analysis, by supporting two laboratory digital computers for general use, a medium size time-shared digital computer (SEL 810B) for real-time on-line applications, and by providing programming service and technical consultation.

Additional services provided by the Section include consultation on: measurement techniques, signal processing; noise and electro-magnetic interference in data measurement systems; and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

INSTRUMENTATION

Following are selected instrumentation projects undertaken by the Section during the past year. These are chosen from a total of 201 projects, and are representative examples of electronic instrumentation.

(1) Patient Activity Monitoring System. The Section is developing a device which will continuously monitor the movement activity of a human subject and record the activity in a memory contained within the device. The device contains a movement transducer with an inherent threshold, which produces a binary output when the threshold is exceeded. These outputs, defined as units of activity, are counted. At pre-selected constant intervals this count is stored in memory and a new count initiated. The memory can store 256 counts, each of which may be as high as 4095 activity units. If the storage interval is 30 minutes, 5 days of data can be stored. The information is extracted from the device by a specially designed instrument containing a microcomputer. The data can then be printed and/or stored on magnetic tape for further analysis. The monitoring device will be produced in a five cubic inch package and can be worn on the body or any limb; if necessary, several devices can be worn by a single subject. The cost of each device will be less than $200 and the read-out instrument (only one is required) will be less than $500. It is expected that this system can be used in any research project where activity must be monitored (manic-depressives, hyperactive children, etc.).

(2) Amplitude/Time Window Discriminator System. The amplitude and time window discriminator developed last year has undergone extensive testing in five labs this year. The addition of time discrimination to standard amplitude discrimination has improved the processing accuracy of neurophysiological signals, while the multiplexed display of the signal and amplitude discrimination levels has made the instrument easier and faster to use. Additional functional units have been designed to support the discriminator, so that a typical system also includes a variable-gain, variable-bandwidth input amplifier, raster and intensity display generators, and a rate meter. Development of a histogram display unit with calculating capability is planned.

(3) High-Speed Microelectrode Amplifiers. Two special-purpose amplifiers were developed to increase the frequency response obtained from high-impedance recording microelectrodes. One design utilized a floating input-stage power supply that was bootstrapped at unity gain by a second-stage amplifier. This resulted in greater than a factor of ten decrease in amplifier input capacitance. For higher-capacitance microelectrodes, the second design utilized a multiple amplifier configuration to present a high input impedance at low frequencies, and a low impedance at higher frequencies. The low impedance improves the high frequency response by shunting the electrode capacitance. At these higher frequencies, the cell must supply current through the microelectrode but the need for capacitance neutralization is eliminated.

(4) Voltage Controlled Current Source and Recording Amplifier for

Micropipettes. The device which allows linear voltage control of current passed through a micropipette, originally designed and published by the Section, has been extensively redesigned to take advantage of advances in the electronics state-of-the-art. Low noise, low voltage drift amplifiers have been added, as have gain adjustments and a calibrated DC input which supplements the signal input. More than twenty of these devices have been built and delivered to investigators in the IRP.

(5) Multi-Channel Voltage-Controlled Current Sources for Drug Electrodes. The standard current source described above has been adopted to multibarrel micropipettes to be used in iontophoretic drug application studies. Use of high-voltage amplifiers gives the current sources a high voltage compliance to pass relatively large currents through very high resistance pipettes. Of primary importance in this application, is the ability of this design to provide a current monitor output that gives a true current reading even when the large voltage compliance of the circuit is exceeded. Both a five and a four channel instrument have been built, with the latter including a multiple trigger unit for pulsed applications.

(6) Selective Detection of Animals at a Specific Location. This system was designed to provide automatic detection of a specific animal at a feeding station. Each of five beagle puppies carries a low power, battery operated, crystal controlled transmitter weighing 5 grams and shaped as a 1.5cm. dia. cylinder, 3cm. long. Each transmitter operates on a separate carrier frequency in the 25 to 27 MHz band. Each frequency is monitored by a separate receiver, and activity on that frequency, indicating the presence of a specific puppy at the feeding station, causes a once per second pulse to appear on an event recorder, on the channel corresponding to that puppy.

(7) High Gain, Low Noise Amplifier. Operational amplifiers were selected to construct a three stage non-inverting amplifier which optimizes the trade-off between bandwidth and noise. The amplifier has a selectable gain of 10, 100 or 1000 and a bandwidth (1db down) of at least 100 KHz at every setting. The noise at the output ranges from 6 millivolts for a gain of 10, to 120 millivolts for a gain of 1000. The main component of this noise is 60 Hz noise from the power supply.

(8) Micromarker. A device to mark the position of an intramuscular electrode for identification during biopsy examination was developed. The device is basically a current source which passes iron ions into the muscle. The rate of deposit, and hence the total accumulation, is controlled by the investigator.

(9) Parallel/Serial Converter. This instrument was designed to convert six decades of parallel BCD formatted data to serial form for output on a single polygraph channel. The output is coded by the pulse height in order to distinguish among a start pulse, a logical "1", and a logical "0". The system has both an automatic and a manual mode. In the automatic mode it can sample and convert the parallel input data at selectable intervals between thirty seconds and ten minutes. The unit can also be controlled externally from a front-panel switch.

(10) Real-Time Clock. This circuit was designed to be used as a peripheral device for a battery powered microprocessor system to control evoked response studies in a field location. The circuit contains a 1 KHz crystal controlled clock and 16 binary stages of division, allowing accurate timing of events up to 65 seconds with one millisecond resolution. The circuit was constructed of 17 CMOS integrated circuits, and uses less than five milliwatts of power in the active mode.

COMPUTER SERVICES

The Section on Technical Development undertook a systems study to determine the best means of utilizing recent advances in minicomputer technology and reductions in cost. The purpose of the study was to provide the laboratory scientist with a low cost, versatile system having real-time, on-line, data acquisition and processing capabilities and the ability to monitor and control an experimental apparatus. Two additional criteria were set. One, that the scientist may perform his experiment independent of any computing facility outside of his laboratory and to have a convenient means of relatively high speed, random access bulk storage for the results of a single experiment. Secondly, that the manufacturer supply an integrated package of hardware and software to minimize the time required for programming and interfacing experiments. This software must be oriented to the laboratory scientist.

The cost of minicomputers has dropped significantly, enough to put them within the budget of the individual laboratories, but the cost of peripherals such as line printers, magnetic tapes, disc storage devices and plotters remains high. These devices are not usually essential to the conduct of an experiment and may be used later, off-line. A cost effective means of providing these services is to locate them in a support facility in each of the areas of major use, the Clinical Center and Building 36. Each support computer will be provided with a full range of peripherals necessary for bulk storage and output of data, and will provide a means for extensive data processing. The individual laboratory computers will have a full range of scientific input and output devices such as analog-to-digital converters, digital-to-analog converters, a real-time clock, digital I/O and an oscilloscope controller. The laboratory computers will have sufficient memory to operate efficiently with higher level languages and will be provided with a low cost means of removable bulk storage which may be read by the support computer. The laboratory computers will also be connected to the support computer by a telephone linkage. All computers will be able to access the facilities of the Division of Computer Research and Technology by telephone, making their large computers available for sophisticated data processing. The resulting distributed network will offer a full range of computer capability to the scientist at lowest cost. The average cost for a laboratory computer will be less than $20,000.

By standardizing on one computer mainframe, STD will be able to maintain a library of modular routines specific to the needs of the IRP with a minimal investment of manpower. Another factor which was considered during the study was the availability of a line of microcomputers which are compatible with, and programmable from, the support computers. Exploitation of the microcomputers should offer improved technological services in the future with large reductions in cost.

On the basis of this study, the Section has elected to standardize on the Digital Equipment Corporation's PDP-11 series. Three support computers, PDP-11/40, are being purchased for installation in Bldg. 36, Bldg. 10, and Poolesville. In addition, five PDP-11/10 computers are being purchased for use in individual laboratories in NIMH and NINCDS. Delivery is expected early in FY76.

The satellite network will phase out the existing multiprogramming facility in the Clinical Center by the end of FY76. The present two minicomputers (PDP-12 and Spear microLINC) operated by STD will be dedicated to specific laboratory applications and the Bldg. 36 support computer will replace the open shop facility presently provided by the PDP-12.

Computer Utilization

PDP-12

		Hours
NINCDS:	Laboratory of Neurophysiology	610
	Applied Neurological Branch (C&FR)	624
NIMH:	Laboratory of Neurophysiology	100
	Section on Technical Development	32
NICHD:	Behavioral Biology Branch	256
	TOTAL	1622

MicroLINC-300

		Hours
NINCDS:	Laboratory of Experimental Neurology	40
	Laboratory of Biophysics	200
	Medical Neurology	200
NIMH:	Section on Technical Development	80
	TOTAL	520

SEL 810B

		Hours
NIMH:	Laboratory of Psychology and Psychopathology	1250
	Adult Psychiatry Branch	1800
	TOTAL*	3050

---

*The SEL 810B is a time-shared computer. The real-time total is less than this figure.

ENGINEERING and FABRICATION

This table shows the distribution of the Section's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Neuro-Otolaryngology, NINCDS	2319	7.94
Adult Psychiatry, NIMH	2297	7.87
Computer Services, NIMH & NINCDS	2026	6.94
Brain Evolution & Behavior, NIMH	2009	6.88
Behavioral Biology, NICHD	2002	6.86
Clinical Science, NIMH	1937	6.64
Biophysics, NINCDS	1899	6.50
Developmental Psychology, NIMH	1887	6.46
Neurophysiology, NINCDS	1857	6.36
Neuropharmacology, St. E's, NIMH	1718	5.89
Psychology and Psychopathology, NIMH	1145	3.92
Surgical Neurology, NINCDS	1007	3.45
Technical Development, NIMH/NINCDS	955	3.27
Clinical Psychobiology, NIMH	947	3.24
Molecular Biology, NINCDS	832	2.85
Neuropathology & Neuroanatomical Sciences, NINCDS	654	2.24
Neurophysiology, NIMH	519	1.78
Neurobiology, NIMH	505	1.73
Neurochemistry, NIMH	437	1.49
General & Comparative Biochemistry, NIMH	401	1.37
Perinatal Physiology, NINCDS	373	1.28
Medical Neurology, NINCDS	340	1.16
Experimental Neurology, NINCDS	322	1.10
Neurochemistry, NINCDS	301	1.03
Extramural Research, NIMH	178	.61
Socio-environmental Studies, NIMH	112	.38
Neural Control, NINCDS	109	.37
Cerebral Metabolism, NIMH	103	.35
NIMH (Total)	15,926	54.56
NINCDS (Total)	11,263	38.59
NICHD (Total)#	2,002	6.85
TOTAL*	29,191	100.00

---

*The time of the Section Chief is not included in the foregoing table.

#NICHD loans the Section one position, and is thus entitled to 2000 hours of service.

[illegible] 3

Libra
Nation
Building
Bethesda, Maryland 20014

DATE DUE

GAYLORD
PRINTED IN U.S.A.

http://nihlib

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
4 0129 1358

WERT
BOOKBINDING
MIDDLETOWN, PA.
NOVEMBER 75
We're Quality Bound

NIH LIBRARY
3 1496 00181 0087